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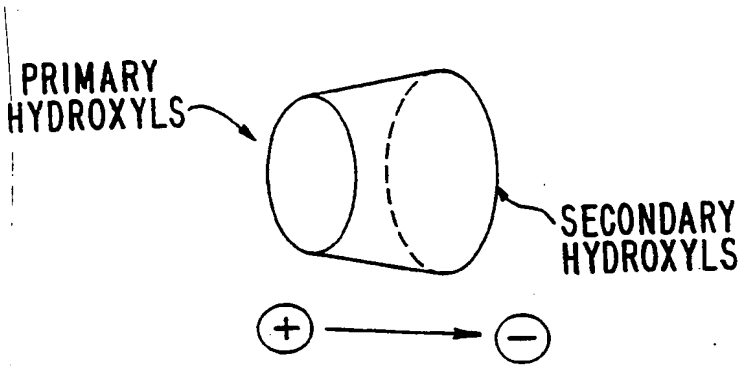

(57) Abstract

Cyclodextrin derivatives and inclusion complexes are provided, as well as intermediates and syntheses for their production. Also provided are methods for designing inclusion complexes to achieve enhanced properties. Pharmaceutical compositions including *inter alia*, cimetidine, amiodarone, polypeptides and piroxicam are also provided, as well as numerous processes which can employ the cyclodextrin derivates disclosed.

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CYCLODEXTRIN COMPOSITIONS AND METHODS FOR PHARMACEUTICAL AND INDUSTRIAL APPLICATIONS

BACKGROUND OF THE INVENTION

The subject matter of this application relates to that of PCT Application AU89/00359, published as WO 90/02141 on 8 March 1990 (hereinafter "the '359 Application"), the disclosure of which is expressly incorporated herein by reference. This application also relates to Australian provisional application Nos. PJ 8899 filed 2 March 1990, PJ 8993 filed 8 March 1990, PJ 9344 filed 28 March 1990, PJ 9373 filed 29 March 1990, PJ 9756 filed 23 April 1990, PK 1538 filed 3 August 1990, PK 1755 filed 16 August 1990, PK 2269 file 12 September 1990, PK 3596 filed 29 November 1990, PK 3624 filed 30 November 1990, PK 4284 filed 21 January 1991, PK 4603 filed 14 February 1991, and PK 4856 filed 27 February 1991, the disclosures of which are also expressly incorporated herein by reference, and to which priority is claimed.

This invention concerns cyclodextrins, cyclodextrin derivatives, inclusion complexes and compositions containing same, methods for their preparation, and various uses thereof. For a detailed discussion of the background of cyclodextrins, the reader is invited to review the "Background of the Invention" section of the '359 Application and the publications cited therein. A brief review is also provided below.

Cyclodextrins (which are cyclic oligosaccharides) have been the subject of increasing interest for more than 25 years. This interest is primarily due to the ability of cyclodextrin molecules to form "inclusion complexes" with other molecules called "guests." A cyclodextrin molecule contains an apolar/hydrophobic cavity or annulus which can "include" an apolar/hydrophobic portion of a guest molecule. While a cyclodextrin's cavity is hydrophobic, the remainder of the molecule is relatively hydrophilic, and thus a cyclodextrin has the potential to include a relatively hydrophobic guest and render it soluble in water.

Of the three most common cyclodextrins, α, ß and γ (6, 7 and 8 D-glucopyranosyl residues, respectively), however, the one which is the most desirable for many guest molecules, ß-cyclodextrin, is also the least soluble. Hence, there have been many efforts to modify cyclodextrins, and especially ß-cyclodextrin, to improve solubility. Likewise, because the usefulness of a cyclodextrin for many applications is a function of how well it keeps the guest molecule included in the annulus, there has been a search for cyclodextrin derivatives, including covalently linked cyclodextrins, which are capable of achieving inclusion complexes of greater stability. In part to address such concerns, the '359 Application provided, *inter alia*, many new cyclodextrin derivatives and processes, as well as synthetic pathways and intermediates for preparing such derivatives.

Despite the ability to selectively modify cyclodextrins, however, there remains a continuing need for guidance in designing complexes in which the cyclodextrin derivative is tailored to the guest molecule such that the one or more substituents on the cyclodextrin exerts a desired effect. There is also a continuing need for cyclodextrin derivatives, intermediates, processes and synthesis useful in providing such inclusion complexes and other compositions.

There is also a continuing need for ways to improve the delivery of molecules which have heretofore proven difficult to deliver. For example, it would be desirable to enhance the delivery of peptides. In recent years, there has been a tremendous increase in the number of products and potential products containing polypeptides, especially proteins, intended for therapeutic, diagnostic or analytical use. Typically, the proteins or other peptides are formulated in aqueous solution for injection or for use in diagnostic or analytical applications. Unfortunately, proteins often suffer from solubility and/or stability problems. Some proteins, for example, are not water-soluble. Others are soluble in water but still suffer from stability problems caused by protein degradation/denaturation,

dimerization and/or polymerization, and the like, any of which may lead to inactivation. This seriously limits shelf-life and often imposes low-temperature storage requirements and restrictions on mechanical movement.

There are numerous causes of protein/peptide instability and degradation, including covalent bond reactions, such as hydrolysis, and the denaturation process. Either of these end results entails diminution or loss of biological activity. Polypeptides are even more difficult to deliver orally. In addition to the hydrolysis and denaturation which can occur in the acidic environment of the gastrointestinal tract, there are numerous enzymes in the mouth (i.e., saliva), lungs, at the surface and within cells such as polymorphic nuclear leukocytes, macrophages and other cells. Also, within the cytoplasm of most cells are destructive mechanisms. For a summary of the causes of protein instability, and the various excipients which have been used with polypeptides, see Wang et al., *Journal of Parenteral Science and Technology*, Technical Report No. 10, "Parental Formulations of Proteins and Peptides: Stability and Stabilizers," Supplement Vol. 42, No. 25, 1988, pp. S3-S26. The skilled artisan will readily appreciate that the aforementioned problems associated with delivering polypeptides can be equally applicable to many other pharmaceutical agents.

The use of cyclodextrins to deliver peptides is disclosed in International Patent Application PCT US89/04099 to Cetus Corporation, which was published 19 April 1990 under International Publication No. WO 90/03784. The disclosure of the Cetus Application is expressly incorporated herein. In particular, the Cetus application discloses the use of various cyclodextrins including hydroxypropyl cyclodextrins to solubilize and/or stabilize various polypeptides and especially proteins. Such cyclodextrin compounds, however, are limited in design and can, in some instances, present certain drawbacks with respect to solubility at low cyclodextrin concentration levels and/or low stability constants such that there remains a need for finding

other delivery vehicles which can enhance the delivery of polypeptides, including proteins.

There is also a continuing need to enhance the delivery of many other pharmaceuticals and industrially useful molecules which possess one or more undesirable delivery attributes. Such other molecules include drugs such as, for example, amiodarone, a broad spectrum antiarrhythmic having several undesirable traits. For example, because of its low water wettability and solubility, amiodarone HCl was only administered orally, until very recently when an injectable solubilized by polysorbate 80 became available. When given orally, absorption is low and erratic and, therefore, its bioavailability is unpredictable. When given intravenously, the recommended administration is by slow infusion in a dilute solution. Only in extreme clinical emergency, may the drug be given as a IV bolus injection over one to two minutes, but this is generally met with poor tolerance. A massive precipitate commonly occurs at the interface of the injection site and hence the risk of embolism becomes significant. The slow dissolution of the precipitate may also expose the patient to transient high drug levels, and subsequent reduction in blood pressure. Circulatory collapse may occur, and temporary hot flashes, sweating and nausea have also been reported. Most of these adverse effects are also related to dosage and duration of therapy, concurrent use of other antiarrythymic agents, severity of the underlying disease state, and wide individual variation in the pharmokinetic profile of the drug. Accordingly, there is room for improvement in both oral and intravenous formulations of amiodarone.

As another example, there is also a continuing need to enhance the delivery of the drug Piroxicam, which is slightly soluble in water (approximately 30 mg/dm^3 in water at pH 5 at 37°C) and dissolves slowly as a consequence of its poor wettability. In therapeutic usage it has a side effect which causes irritation of the gastro-intestinal tract. A substantial proportion of this side effect is considered to arise from direct

contact between solid Piroxicam and the walls of the gastro-intestinal tract.

Further, it has been a goal for many years to be able to target the delivery of certain pharmaceutical or assay agents to particular areas of the body such as tumors. One currently accepted method for such targeting has been the use of monoclonal antibodies or fragments thereof which are specific to the tumor in question. Difficulties can arise, however, in conjugating the pharmaceutical or assay agent to the antibody or fragment. The covalent bond between the agent may degrade the bioactivity of the agent or the antibody. The agent may have more than one reactive group which is susceptible to covalently bonding to the antibody or to other molecules of the agent, resulting in a crosslinking of agent molecules and antibody. Moreover, if the conjugation breaks, the agent may be free to target healthy cells and thereby injure the patient. Similarly, there is a need for ways to conjugate pharmaceuticals to other targeting agents such as hormones, lymphokines such as interleukins, or other carriers.

Further still, many pharmaceutical agents can incite an immune response in a patient. For example, penicillin in some individuals is antigenic, giving rise to anti-penicillin antibodies. This can pose two adverse consequences. First, if penicillin is given to an allergic individual, a severe reaction may occur which can vary from a skin rash or asthma to anaphylaxis and death. Second, the drug does not react at its site of activity because it is sequestered by antibody. Other pharmaceutical agents which may be potentially immunogenic are antibodies themselves, peptides or other synthetic or natural materials.

There also remains a continuing need for enhancement of other drug delivery systems. For example, liposomes are a well known means of drug delivery. However, problems can arise if a drug to be incorporated into the liposome has low solubility or wettability, or if it is susceptible to instability, e.g., upon

storage. Likewise, there are continuing needs for providing enhanced, prolonged delivery of pharmaceuticals in the body by using mechanical means which meter amounts of the agent to the body, and surgically implanted matrices or "disks," e.g., for releasing constant amounts of agents which prevent pregnancy, or simply by surgically implanting a long acting form of the compound. Nevertheless, there is always a need for improved compositions and methods for providing prolonged release.

Difficulties with delivery of bioactive compounds are not unique to pharmaceuticals. For example, a continuing problem with applying some herbicides and pesticides to plants is that they evaporate too quickly after application to allow the bioactive molecule to be effective. Thus, there is a continuing need for enhanced delivery of other active agents such as herbicides, pesticides and agricultural agents such as fertilizers.

On a different topic, there are certain physiological conditions, e.g., epilepsy, which can be caused by metabolic disorders associated with compounds produced *in vivo* through enzyme catalysis. Other physiological conditions can result from the presence of unwanted proteins or toxins in the body. Thus, there is a continuing need for compositions and methods which are useful in preventing or decreasing the occurrence of certain products of enzyme-catalysis, or which can diminish the effective concentration of, or remove unwanted proteins or toxins from a patient.

SUMMARY OF THE INVENTION

Building upon the disclosure of the '359 Application, this specification provides, *inter alia,* cyclodextrin derivatives, inclusion complexes, processes, syntheses and intermediates which are useful in the application of cyclodextrins to, *inter alia,* pharmaceutical (diagnostic and therapeutic) and industrial applications.

Accordingly, a first embodiment provides a method for designing cyclodextrin inclusion complexes comprising a useful agent and an otherwise substituted or unsubstituted cyclodextrin or two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group. The process comprises first determining whether the useful agent possesses at least one group capable of non-covalent association. Next, the orientation of the agent in the cyclodextrin annulus and the relative position of the associable group is determined by considering the dipole moment of the agent and the position of hydrophobic or apolar groups on the agent. The final step is to selectively substitute at least one substituent comprising a group which will associate with the associable group on the agent onto one or more C2, C3 or C6 positions of the cyclodextrin or linked cyclodextrins. The substituent(s) should be configured in order to position its associable group(s) in the approximate vicinity of the associable group on the agent to promote association therebetween.

Where the inclusion complex comprises two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, the process may further comprises the steps of: (1) determining the dipole moment of each individual cyclodextrin of the linked cyclodextrins; and (2) linking the cyclodextrins such that the dipole moments align where possible.

Also provided is a broad range of cyclodextrin derivatives and inclusion complexes, including cyclodextrin derivatives comprising groups which are charged, polar or are capable of forming a non-covalent association with another group. The inclusion complexes can comprise an active agent such as a pharmaceutical, herbicidal, pesticidal, agricultural, cosmetic or personal care agent. Synthetic procedures for preparing such derivatives are also provided.

Notable among such derivatives are those having the formula CD - W - R - L , wherein

CD represents an otherwise substituted or unsubstituted cyclodextrin,

W represents a functional linking group, such as amino, amide, ester, thioether, thioamide, thioester or thiol,

R represents a substituted or unsubstituted group, and

L represents a group selected from reactive, charged, polar or associating groups such as amino, carboxyl, hydroxyl, sulfonate phosphate, acyloxy, alkyloxy or thiyl. Such compounds are useful both as delivery vehicles and as intermediates for preparing other compounds.

Also notable are the compounds of the formula CD - X - R - Q, wherein:

X represents $-\underset{\underset{R'}{|}}{N}-\overset{\overset{O}{\|}}{C}-$ or $-S-\overset{\overset{O}{\|}}{C}-$

R and R' represent substituted or unsubstituted groups, and R' can represent hydrogen, and

Q is a carboxylic acid group or a carboxylic acid group derivatized to undergo substitution. Such compounds are very advantageous as delivery vehicles when Q is a carboxylic acid, and as intermediates when Q is a carboxylic acid group derivatized to undergo substitution.

Also provided are cyclodextrin derivatives comprising first and second otherwise substituted or unsubstituted cyclodextrins covalently bonded together by at least one linking group which links a C2, C3 or C6 carbon on the first cyclodextrin to a C2, C3 or C6 carbon on the second cyclodextrin. Many variations of the linked cyclodextrins are provided, and especially asymmetrical linked cyclodextrins in which the cyclodextrins are different, the links contain different functional groups and/or the first cyclodextrin is linked through a C6 carbon to a C2 or C3 carbon of the second cyclodextrin.

Another embodiment provides cyclodextrin derivatives in

which an otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins, are covalently bound to a useful agent such that the covalent bond, when broken, will yield the agent in an active form.

Other embodiments provide cyclodextrin derivative comprising an otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins that are covalently bonded to a carrier which can target cells of interest within a patient. Such carriers can comprise, for example, an antibody or fragment thereof, hormones or lymphokines.

Other embodiments provide otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins covalently bonded, physically entrapped or encapsulated, or otherwise associated with a carrier useful for localized or prolonged delivery of a useful agent. Such carriers can include, for example, liposomes, solid matrices,etc.

Other embodiments include processes in which cyclodextrins in accordance with this invention may be used. Included are processed for masking the taste of a pharmaceutical agent, for increasing the solubility of a pharmaceutical or other useful agent, for targeting a pharmaceutical agent to a selected group of cells, for encapsulating into a liposome a pharmaceutical agent of relatively low solubility or wettability, for improving the stability of a pharmaceutical agent that is encapsulated in a liposome, for decreasing or preventing an immunogenic reaction of a patient to a pharmaceutical agent, for the treatment of metabolic disorders associated with compounds produced *in vivo* through enzyme catalysis, and for treating a patient suffering from an excess of a toxin.

Also provided are methods for preparing the compounds in

accordance with this invention, including intermediates and linked cyclodextrin derivatives.

Further still, many pharmaceutical composition are provided, including, *inter alia* advantageous composition for the delivery of cimetidine, peptides, amiodarone and piroxicam.

Accordingly, it is an object of this invention to provide methods for designing inclusion complexes and prodrugs to provide complexes and prodrugs having advantageous stability and/or solubility features.

It is another object of this invention to provide cyclodextrin derivatives and inclusion complexes, including cyclodextrin derivatives substituted with groups which are charged, polar or are capable of forming a non-covalent association with groups on an included guest.

It is another object of this invention to provide cyclodextrin derivatives which are useful as intermediates for preparing other cyclodextrin derivatives, or which are useful for preparing inclusion complexes and compounds in which a useful agent such as a pharmaceutical is covalently bonded to a cyclodextrin, such that the covalent bond, when broken, will yield the agent in active form. In this regard, it is also an object to provide synthetic procedures for preparing cyclodextrin derivatives in accordance with this invention.

Yet another object of this invention comprises the formation of advantageous inclusion complexes and prodrugs comprising cyclodextrin derivatives and pharmaceutically active compounds such as cimetidine, polypeptides, amiodarone and piroxicam.

Yet another object of this invention is to provide many useful processes which can employ cyclodextrin derivatives in accordance with this invention.

Additional objects and advantages of the invention will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and the advantages of this invention may be realized and obtained by means of the compositions of matter and processes particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the dipole moment of a cyclodextrin molecule;

Figure 2 illustrates the dipole moment of the cyclodextrin host and guest molecule in an inclusion complex;

Figure 3 illustrates the equilibrium between a cyclodextrin host and guest molecule;

Figure 4 illustrates a cyclodextrin inclusion complex;

Figure 5 illustrates a possible equilibrium for an inclusion complex comprising a guest molecule and cyclodextrins which are linked by their primary carbons;

Figure 6 illustrates a possible equilibrium for an inclusion complex comprising a guest molecule and linked cyclodextrins in which a primary carbon of one cyclodextrin is linked to a secondary carbon of another cyclodextrin;

Figures 7A-7C illustrate additional types of inclusion complexes which can be prepared in accordance with this invention;

Figure 8 illustrates the hydrolysis of BTEE and BTEE + β-cyclodextrin by α-chymotrypsin;

Figure 9 illustrates a semilogrithmic graph of the mean plasma concentration of amiodarone obtained after intravenous bolus injection of Cordarone X (open circle) and amiodarone-β-CDNSc complex (close circle);

Figure 10 illustrates a semilogarithmic graph of the plasma concentration of amiodarone for dog 10 obtained after intravenous bolus injection of Cordarone X; and

Figure 11 illustrates a semilogrithmic graph of the plasma concentration of amiodarone for dog 12 obtained after intravenous

bolus injection of Cordarone X.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

The following definitions are provided for some basic terms that are used throughout this specification.

Cyclodextrin - refers to α-, β-, γ-, or δ-cyclodextrins, which are those that are generally available. It will be appreciated, however, that if other cyclodextrins are discovered or become available in sufficient commercial quantities, such cyclodextrins shall also be encompassed by this invention.

Cyclodextrin Derivative - refers to a cyclodextrin-containing compound in which one or more atoms or groups of atoms are substituted for a C2, C3 or C6 hydroxyl or hydroxyl hydrogen, i.e., "modified cyclodextrins." The term cyclodextrin derivative also encompasses "linked cyclodextrins" where two or more cyclodextrins are linked together, and compounds where a useful agent such as a pharmaceutical is covalently bonded to a cyclodextrin, such that the covalent bond, when broken will yield the agent in active form. This term also includes any salt or hydrate which can be formed from the cyclodextrin derivative.

Modified Cyclodextrin - refers to a species of cyclodextrin derivatives that contains one or more atoms or groups of atoms substituted for a C2, C3 or C6 hydroxyl or hydroxyl hydrogen. The term modified cyclodextrin will not be meant to include compounds where two or more cyclodextrins are linked together, or compounds where a useful agent such as a pharmaceutical is covalently bound to a cyclodextrin. This term also includes any salt or hydrate which can be formed from the modified cyclodextrin.

Linked Cyclodextrins - refers to two or more cyclodextrins linked together by one or more bridging groups.

The bridging groups can link a C2, C3 or C6 position of one cyclodextrin to any one of the C2, C3, or C6 positions of the other cyclodextrin. This term includes any salt or hydrate which can be formed from the linked cyclodextrins.

Prodrug - refers to a cyclodextrin derivative in which a pharmaceutical agent is covalently bonded to a substituted or unsubstituted cyclodextrin or to two or more linked cyclodextrins such that the covalent bond, when broken, yields the agent in active form. The product formed by this reaction comprises the residue of the pharmaceutical linked to a cyclodextrin, or to a pendant arm substituted thereon, through a linking group that is formed by the reaction of a functional group on the pharmaceutical agent with a functional group on the cyclodextrin or pendant arm. Thus, the residue of a pharmaceutical agent can be covalently bonded to the cyclodextrin through a linking group that is substituted for a C2, C3 or C6 hydroxyl or hydroxyl hydrogen, or it can be covalently bonded via a pendant arm that is substituted to a C2, C3 or C6 position. This term also includes any salt or hydrate which can be formed from the prodrug.

Cyclodextrin Inclusion-Association Complex - refers to an inclusion complex in which there are one or more associable groups or portions of a group located on a substituent that is substituted at a C2, C3 or C6 position of a cyclodextrin, which groups or portions form an association with one or more associable groups or portions of a guest atom or molecule. The associable portions can include polar or charged groups or portions, or groups or portions capable of hydrogen bonding. This term also includes any salt or hydrate which can be formed from the inclusion-association complex.

Cyclodextrin Inclusion Salt - refers to an inclusion-association complex in which the associable group or portion of the cyclodextrin substituent carries a net positive or negative charge which causes it to associate with an oppositely charged

group or portion of a guest atom or molecule. This term also includes any other salt or a hydrate which can be formed from the cyclodextrin inclusion salt.

Pharmaceutical Agent - refers to compounds or their salts or hydrates which have a pharmaceutically recognized use. Pharmaceutical shall also be taken to mean drug, isotope, toxin or any other molecule used either to detect, i.e., diagnose, or treat pathological lesion, e.g., cancer, or to prevent the occurrence of such a lesion. It will be appreciated that the term is not intended to cover compounds which have some type of bio-affecting activity, but which are not recognized as pharmaceutical agents by the medical community or drug regulatory agencies. For example, a dye is not considered to be a pharmaceutical agent, even though it might possess some type of bio-affecting activity. Similarly, the term "pharmaceutical agent" does not include unreacted reactants, other products, or solvents (including water) that are present in the reaction mixture when synthesizing cyclodextrin derivatives or which are present when forming inclusion complexes.

Pesticidal, Herbicidal, Agricultural, Cosmetic or Personal Care Agent - refer to compounds or their salts or hydrates which have a generally recognized use in the fields. For the purpose of this invention, a dye is not considered to be such an agent. Similarly, these terms do not include unreacted reactants, other products, or solvents (including water) that are present in the reaction mixture when synthesizing cyclodextrin derivatives or which are present when forming the inclusion complexes.

Useful Agent - includes pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic or personal care agents or salts or hydrates thereof as defined above.

Carrier - refers to atoms or molecules to which a cyclodextrin, cyclodextrin derivative and/or inclusion complex

may be bound, entrapped or encapsulated, covalently or otherwise, and which may impart at least one advantageous property to the cyclodextrin, cyclodextrin derivative and/or inclusion complex which is not already possessed thereby. Although it may occasionally be the case, the carrier or portions thereof generally will not be included in the cyclodextrin annulus, and will generally not comprise the useful agent for which delivery is sought by means of the cyclodextrin, cyclodextrin derivative and/or inclusion complex. Rather, the carrier generally will be employed to enhance delivery of the useful agent. For example, carriers can include targeting molecules for pharmaceutical applications such as antibodies or fragments thereof, hormones, cytokines, lymphokines, receptors in soluble form, other pharmaceutical agents, toxins or isotopes or any other agent where one molecule is bonded to the other. The carrier may also comprise a matrix or physical encapsulant (e.g., liposome) with which the cyclodextrin, cyclodextrin derivative and/or inclusion complex may be physically associated.

Solubility - refers to solution in water or other aqueous-based media.

II. DESIGNING INCLUSION COMPLEXES AND PRODRUGS

The '359 Application provided guidance in tailoring cyclodextrin derivatives to enhance the effect of substituents upon the inclusion complex. For example, based on the estimated orientation of the guest in the cyclodextrin annulus, the cyclodextrin can be substituted with associable groups or pendant arms containing associable groups, e.g., polar, charged or capable of forming other associations, which can exert a desired effect upon associable groups on the guest molecule. Other factors, however, may also affect the orientation of the guest in the inclusion complex.

For example, many molecules have a dipole moment; that is, their atoms and their electrons and nuclei are so arranged that one part of the molecule has a positive electrical charge while

the other part is negatively charged. The molecule thus becomes a small magnet or dipole. Cyclodextrins have a dipole moment resulting from a two-fold greater number of secondary hydroxyl oxygens than primary hydroxyl oxygens. The dipole moment of a cyclodextrin is illustrated in Figure 1.

In an inclusion complex, therefore, an included species having a dipole moment will possess a tendency to orient itself so that the positive end of the included species will be in the vicinity of the secondary hydroxyls and the negative end will be in the vicinity of the primary hydroxyls. This phenomenon is illustrated in Figure 2. The skilled artisan will appreciate that the inclusion complex of Figure 2 is actually only part of the overall equilibrium equation illustrated in Figure 3, the amount of included species being dependent on the equilibrium or stability constant K. The inclusion constant K is defined by the equation:

$$K=[\text{included species}]/[\text{non-included species}]$$

or

$$K=[\text{included species}]/[\text{cyclodextrin}][\text{free molecule}].$$

When designing inclusion complexes, one can thus take in account, and indeed take advantage of the dipole moment phenomenon. Since the included species will tend to achieve the equilibrium shown in Figure 3, the substitutions to the cyclodextrin can be tailored to achieve maximum effect. This method of design can be particularly important in instances where the guest theoretically is capable of more than one orientation within the cyclodextrin annulus. Thus, for example, substitutions for the primary hydroxyls can be made so that they will exert a desired effect on the portion of the included molecule expected to be present in that vicinity. Similarly-intended substitutions for the secondary hydroxyls can also be made. This method of inclusion complex design is generally illustrated in Figure 4. Whether to substitute for primary or secondary hydroxyls thus becomes a function of the molecule to

be included, and the basic techniques for selectively achieving desired primary and secondary substitutions are provided in the '359 Application.

Moreover, knowing that the cyclodextrin is negatively polarized in the vicinity of the secondary hydroxyls, it may be possible to enhance the overall dipole of the cyclodextrin derivative by substituting the secondary carbons with stronger electronegative groups, or substituting the primary carbons with weaker electronegative groups (e.g., NH_2). This can thus enhance the attraction with the guest, and thereby enhance one or more properties of the inclusion complex such as stability.

It is also important that the dipole moment be considered when designing inclusion complexes comprising linked cyclodextrins. Figure 5 illustrates cyclodextrins linked by their primary carbons. In the aligned species (designated as A), the dipole moments are in opposing directions, which leads to instability of the linked configuration. This can result in an equilibrium with a differently-configured species such as the staggered configuration (designated as B). That equilibrium lessens the concentration of species A, which in turn decreases the concentration of the inclusion complex C. For the same reason, the inclusion complex C can also have a somewhat unstable configuration because one end of the guest molecule can be in the vicinity of a cyclodextrin having a like charge.

Figure 6 illustrates a linked configuration which may ameliorate the aforementioned problem and thus lead to an inclusion complex having enhanced properties. In Figure 6, a primary carbon of a first cyclodextrin is linked to a secondary carbon of a second cyclodextrin, thereby creating a uniform dipole moment for the entire linked cyclodextrin species. The cyclodextrins should thus remain aligned and facilitate inclusion of the polar molecule. Moreover, the dipole moment of the linked cyclodextrin can complement the dipole moment of the included guest.

While the foregoing discussion is largely devoted to increased interaction between the host and guest, it should also be noted that the desired effect, in some instances, may be a decreased interaction, i.e., a less stable complex. For example, if a cyclodextrin is being used to mask the taste of a pharmaceutical, it may be desirable to provide an inclusion complex which is stable in the mouth but quickly dissociates in the stomach to release the pharmaceutical. The same reasoning can also apply to cyclodextrin derivatives in which an agent is covalently linked to a cyclodextrin such that the covalent bond, when broken, yields the agent in active form, e.g., a prodrug. That is, the covalent bond can be designed to break relatively quickly to provide a fast delivery of the agent.

For example, a bond that is susceptible to acid hydrolysis such as that in an ester or amide, would thus be susceptible to being broken in the acidic environment of the stomach. It has been found that ester linkages are preferred where quick hydrolysis is desired. Alternatively, amide linkages which are part of a pendant arm can also provide relatively quick hydrolysis rates. Amide linkages which are directly substituted onto the cyclodextrin generally posses long rates of hydrolysis and are thus more likely to be useful where release in the intestine is desired. Alternatively, the bond could be susceptible to being broken by other normally occurring internal conditions in the stomach or intestine of a host. Further, the cyclodextrin derivative, e.g., modified cyclodextrin, linked cyclodextrins or prodrug can be further substituted, either on the pendant arm, the linking group, or on another glucopyranosyl residue, with one or more groups which could assist in acid or base catalyzed hydrolysis of such ester or amide bonds. Such reactions could thus be designed either to be pH-dependant reactions or to be catalyzed by, for example, endogenous enzymes or flora found in the particular areas of the body where release is desired. Further still, the composition can contain multiple types of bonds and/or complexes designed to provide release at different rates and/or locations in the patient.

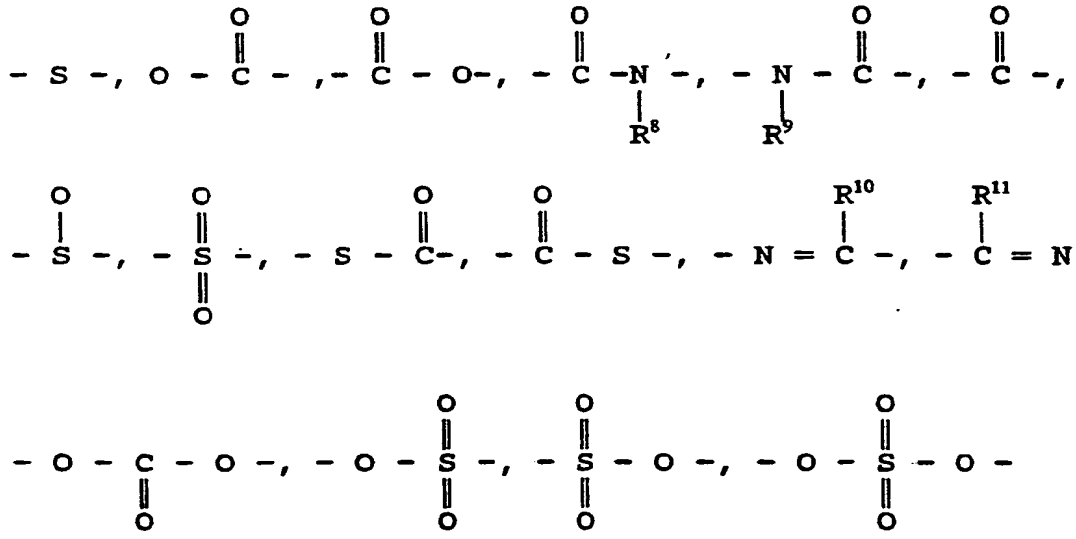
In this regard, Figures 7A to C illustrate inclusion complexes comprising two cyclodextrins wherein each cyclodextrin has one or more substituents which can associate. In Figure 7A, linked cyclodextrins contain associable groups X and Y, which represent charged or polar atoms or groups of atoms, or some other group capable of interaction (e.g., hydrogen bonding). The groups can be selected to provide desired release profiles. For example, the selected groups can be oppositely charged in the acidic environment of the stomach, but one or both could lose its charge (or take on opposite charges) in the relatively neutral environment of the intestines. For example, a histidine or imidazole group may be protonated (and thus positively charged) in the stomach but not in the intestine. By pairing such a group on one cyclodextrin with a negatively charged group such as sulfonate on another cyclodextrin, an association that could form in the stomach may essentially disappear in the intestine. Such design provides a more stable complex in the stomach and a less stable complex in the intestine, resulting in more complexed drug passing through the stomach and more free drug being released in the intestine. It is noted that while Figures 7A to C illustrate only complexes containing one drug molecule that is included through the secondary ends of the cyclodextrins, other configurations such as those shown in the '359 Application (Figures 12D, F and G therein), as well as other variations are certainly possible and are within the scope of this invention. Figures 7B and C illustrate other variations of complexes wherein two otherwise substituted or unsubstituted cyclodextrins contain one or more associable groups. Of course, many other configurations are possible.

III. CYCLODEXTRIN DERIVATIVES

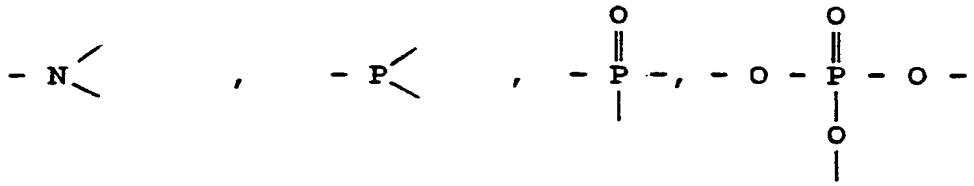
The importance of being able to selectively modify cyclodextrins using the methods described in the '359 Application and hereinafter can thus readily be seen. These procedures can provide the cornerstone for tailoring cyclodextrins to the guest in order to achieve a desired effect.

A. Modified Cyclodextrins

Modified cyclodextrins in accordance with this invention can comprise an otherwise substituted or unsubstituted cyclodextrin in which at least one C2, C3 or C6 hydroxyl is substituted with a group selected from $-XR^1$, YR^3, $SiR^4R^5R^6$, and $-R^7$, wherein X can R^{14} and R^{15} represent groups as defined by R^1-R^{13} above, and represent



Y can represent



and wherein R^1 to R^{11} can each represent the same or different groups selected from: the groups $-XR^1$, YR^3, $SiR^4R^5R^6$, and $-R^7$ are as defined above, hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heterocyclyl, and wherein any two or three groups bonded to the same substituent can be taken together to represent a single group multiply bonded to said same substituent, and wherein R^1 to R^{11} may be further substituted by at least one $-XR^1$, $-YR^2R^3$, $-SiR^4R^5R^6$, $-R^7$, halogen, and OR^{12}, wherein R^{12} is as defined for R^1 to R^{11}.

Cyclodextrins in which one or more C2, C3 or C6 hydroxyls are selectively substituted by ether substituents are also encompassed. For example, cyclodextrin derivatives thus substituted only on one or more secondary carbons, or uniformly substituted only on one, two or three, etc. primary carbons are encompassed. The ether substituents may be further substituted with any of the foregoing groups.

Of the above recited inclusion complexes, preferred groups include the substituted amino cyclodextrins, i.e., cyclodextrins wherein at least one substitution for said C2, C3 or C6 hydroxyl is of the formula $-YR^2R^3$, wherein Y is N, and R^2 and R^3 are as previously defined. Also of particular interest are the inclusion complexes wherein R^2 is hydrogen and R^3 represents amino, hydroxyl, carboxyl, sulfonate (SO_3^-), phosphate (PO_4^{-3}), substituted alkyl, cycloalkyl, or aryl, or wherein R^2 and R^3 are taken together to represent a hereto substituted multiply bonded alkyl group. A discussion of some preferred modified cyclodextrins is provided in the '359 Application.

Also included within the scope of this invention are inclusion complexes in which at least one pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic, personal care or other useful agent is included in a modified cyclodextrin as described above.

Many modified cyclodextrins in accordance with this invention will possess one or more pendant arms as described in the '359 Application. One general formula for preferred pendant arm cyclodextrin derivative are of the formula CD - W - R^{13} - L, wherein

CD represents an otherwise substituted or unsubstituted cyclodextrin,

W represents a functional linking group,

R^{13} represents a group defined the same as R^1-R^{12} above, and

L represents a group selected from reactive, charged, polar or associating groups. Advantageously,

W represents an optional, functional linking group such as amino, amide, ester, thioether, thioamide, thioester, etc.,

R^{13} represents an optional arm such as substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl, and

L represents an optional group selected from reactive, charged, polar or associating groups, e.g., amino, carboxyl, hydroxyl, sulfonate, phosphate, acyloxy, alkyloxy and thiyl.

While cyclodextrin derivatives having at least one of each substituent constitute a preferred group, each of the foregoing groups is optionally present. For example, the reactive, charged, polar or associating species can be bonded directly to the cyclodextrin or to the functional linking group. Likewise, a reactive, charged, polar or associating species may not always be desired. Moreover, such species can be anywhere on the arm, and there can be more than one such species on the arm, e.g, the arm could possess multiple groups that could associate with multiple groups on an included or associated molecule, such as biological molecules which may contain many repeating groups such as amino, carboxyl, and hydroxyl. The arm can also contain other functional or reactive groups which, in turn, may be used to link yet other arms and charged, polar or associating species. Preferred modified cyclodextrins of CD-W-R-L group also include those in which a carboxyl-substituted alkyl group is linked to a C2, C3 or C6 position of the cyclodextrin through an amino, ester, amide, thioether, thioester, thioamide or other functional linking group. Alkyl groups of from 1-3, 1-6, and 1-10 carbons comprise preferred groups. Those of from 10-20 comprise another preferred group, and those of greater than 20 carbons comprise yet another preferred group.

It has been found that, in some instances, advantageous results can be obtained using cyclodextrin derivatives in which at least one C2, C3 or C6 hydroxyl is substituted with a group having a net negative charge, or with a substituent that contains a group having a net negative charge. Examples of groups that

can carry a net negative charge include hydroxyl, carboxyl, phosphate (PO_4^{-3}) or sulfonate (SO_3^{-1}). Other groups having net negative charges will be readily apparent to those skilled in the art.

Preferred modified cyclodextrins of this group include those in which a carboxyl-substituted alkyl group is linked to a C2, C3 or C6 position of the cyclodextrin through an amino, ester, amide, thioether, thioester, thioamide or other functional linking group. Alkyl groups of from 1-3, 1-6, and 1-10 carbons comprise preferred groups. Those of from 10-20 comprise another preferred group, and those of greater than 20 carbons comprise yet another preferred group. One example of such compounds is 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-β-cyclodextrin (hereinafter "β-CDNSc"), which as discussed below, has provided inclusion complexes having advantageous properties. For example, it has been found that by forming an inclusion complex with cimetidine (Tagamet) and β-CDNSc using a 1:1 (cimetidine:β-CDNSc) molar ratio, the solubility of cimetidine in water can be increased to greater than 80 mg/mL. It has also been found that by forming an inclusion complex with amiodarone and β-CDNSc using a 1:2 (amiodarone:β-CDNSc) molar ratio, the solubility of amiodarone in water can be increased by a factor of 35.

The modified cyclodextrin, β-CDNSc, is also an example of another preferred group of cyclodextrin derivatives having the formula CD - X - R^{14} - Q, wherein:

X represents $-N(R^{15})-C(=O)-$ or $-S-C(=O)-$

R^{14} and R^{15} represent groups as defined by R^1-R^{13} above, and

Q is a carboxylic acid group or a carboxylic acid group derivatized to undergo substitution, e.g., acid chloride, acid anhydride or ester. Of these derivatives, those where R^{14} is alkyl comprise a preferred group. Of the alkyl groups, those of from 1-3, 1-6, and 1-10 carbons comprise preferred groups. Those of from 10-20 comprise another preferred group, and those of greater than 20 carbons comprises yet another preferred group.

Preparation of such compounds is described in the '359 Application and also below.

Where Q is a carboxylic acid, the compound can be used either for delivery of drugs such as amiodarone, or as an intermediate in the preparation of other cyclodextrin derivatives, including especially asymmetric linked cyclodextrins and prodrugs. When it is desired to use the derivative as an intermediate, the carboxylic acid advantageously can be derivatized to undergo substitution, for example, by forming an acid chloride, acid anhydride or ester.

B Linked Cyclodextrins

As with the '359 Application, another preferred embodiment of this invention comprises at least two otherwise substituted or unsubstituted cyclodextrins covalently bonded to each other by at least one linking group. The at least one linking group links a first cyclodextrin at a C2, C3 or C6 position to a second cyclodextrin at a C2, C3 or C6 position. The cylodextrins can be substituted as described above. When there are only two cyclodextrins which are not otherwise substituted and are linked by only one linking group, that linking group is other than a disulfide that links the two cyclodextrins at the C6 position.

A preferred group of the linked cyclodextrins are those in which only two cyclodextrins are linked together. Further, from the discussion in Section II above, one can also see the importance of linked cyclodextrins in which a first otherwise substituted or unsubstituted cyclodextrin is linked through one of its primary (C6) carbons to a secondary (C2 or C3) carbon of a second otherwise substituted or unsubstituted cyclodextrin. Thus, otherwise substituted or unsubstituted cyclodextrins which are linked by C6-C3 or C6-C2 linkages comprise yet another preferred embodiment of this invention. As discussed below, preparation of such asymmetrical linked cyclodextrins is greatly facilitated by the above-described intermediates of the formula CD-X-R-Q.

Also, as discussed in the '359 Application, the linked cyclodextrins are preferably linked by at least one linking group of the formula - X - R^{16} - Y - or - R^{17} - , wherein

X and Y can be the same or different, and represent functional linking groups, and

of the formula - X - R^1 - Y - or - R^2 - , wherein

X and Y can be the same or different, and represent functional linking groups such as ether, thioether, ester, thioester, amide, thioamide, and amine, and

R^{16} and R^{17} represent groups as defined by R^1-R^{15} above, and are advantageously selected from substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl.

The otherwise substituted or unsubstituted cyclodextrins which are linked may also be the same or different. For example, an α-cyclodextrin can be linked to a β-, γ- or δ-cyclodextrin. Such an asymmetrical configuration may be advantageous when the guest contains multiple hydrophobic groups of different size. Moreover, the length of the linking group may be varied to accommodate guest molecules of different sizes.

Also included within the scope of this invention are inclusion complexes in which at least one pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic, personal care or other useful agent is included in a modified cyclodextrin or linked cyclodextrins as described above.

C. Cyclodextrin Derivatives Having a Covalently Bonded Agent (e.g., Prodrugs)

In another preferred embodiment of this invention, an otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins, can be covalently bonded to a useful agent such as a pharmaceutical, herbicidal, pesticidal, agricultural, cosmetic or personal care agent, wherein the covalent bond, when broken, will yield the agent in an active form. The modified and linked

cyclodextrins which can be used include those described above and in the '359 Application. Guidance in preparing such compounds is also provided in the '359 Application. Upon reading this disclosure, the skilled artisan will also appreciate that the intermediate CD-R-X-Q can be very helpful in preparing such derivatives.

A preferred embodiment of such derivatives comprises a pharmaceutical agent covalently bound to a cyclodextrin, i.e., a prodrug, wherein the covalent bond will be broken down in the normally occurring internal activity of a host animal.

D. Cyclodextrins and Carriers

As stated above in the "Definitions" section, the term "carrier" refers to atoms, molecules and other structure to which a cyclodextrin, cyclodextrin derivative and/or inclusion complex may be bound, associated or encapsulated, covalently or otherwise, and which may impart at least one advantageous property to the cyclodextrin, cyclodextrin derivative and/or inclusion complex which is not already possessed thereby. Carriers can be categorized into several different groups.

A first group of carriers comprises those atoms or molecules which can be used to target cells of interest within a patient. Examples of such can include antibodies or fragments thereof which are specific to tumors for the therapy of cancer, hormones or interleukins for the therapy of infectious disease, cancer and for the treatment of immune deficiency. In these examples, the cyclodextrin derivative generally first would be conjugated to the carrier by a chemical reaction. Thereafter, the carrier-cyclodextrin conjugate would be exposed to the pharmaceutical agent which would form an inclusion complex with the cyclodextrin. Alternatively, cyclodextrin prodrugs could be covalently attached to the targeting carrier. In addition to the targeting advantage, using such carriers may provide other advantages such as:

(a) the amount of drug which can be conjugated to the carrier

may be able to be increased;

(b) the drug may be in a "protected" form resistant to lysis by extra and intracellular enzymes and other materials;

(c) the integrity of the compounds may be preserved, i.e. both the structure and function of both agent and carrier may be preserved as they will not be exposed to the stringent conditions often required for the conjugation procedures; moreover, cyclodextrins are likely to be resistant to such stringent conditions.

Furthermore, the cyclodextrin derivatives disclosed in the '359 Application and herein lend themselves well to such applications since they may contain very precise substitutions which facilitate linking to the carrier. For example, a cyclodextrin derivative containing a thiol, amino or carboxylic acid group could be reacted with the complementary carboxylic acid amino, or thiol group to form an amide, thioamide, thioester or disulfide covalent bond. Other reactive groups could be employed to form other bonds such as ester and amino. Preferably, the group on the cyclodextrin derivative could be activated to react easily with a complementary group on the antibody or other carrier.

For example, a carboxylic acid group could be derivatized to undergo substitution by forming an acid chloride, acid anhydride or ester, which could then be reacted with an amino group on an antibody to form an amide bond. In this way, the form of the pharmaceutical agent is not altered and can be targeted to the cells of interest in the protected form of the inclusion complex.

This use of a cyclodextrin derivative as a "linking agent" could have many applications, for example:

(a) linking agents such as Melphalan, anthracyclines and other cytotoxic drugs to antibody for their specific carriage to tumors;

(b) linking agents to Interleukins (Il-1 - IL-10) for their

passage to target cells such as tumors and sites of infection;

(c) linking plasmaprotein such as albumin for the selective intravascular delivery of pharmaceutical agents such as drugs, hormones and the like into the circulatory system;

(d) linking diagnostic reagents such as radioactive particles to antibody for the detection of tumors; and

(e) linking to lectins.

Another category of carriers includes atoms, molecules, matrices or encapsulants which are generally useful for localized or prolonged delivery. Examples of such carriers include solid matrices such as disks, and liposomes, although in some instances, liposomes can be targeting carriers as described above. In some instances, the cyclodextrin derivative could be covalently bonded or physically entrapped or encapsulated in the carrier, and the agent would then be included in the cyclodextrin (except where cyclodextrin prodrugs are employed). Alternatively, the inclusion complex may first be formed, and the inclusion complex could then be bonded, entrapped or encapsulated in the carrier.

When liposomes are used as a carrier, the pharmaceutical agent to be incorporated into the liposomes generally can be included either in the buffer if is water soluble, or included in the organic solvent if it is hydrophobic. For agents with low solubility and wettability, cyclodextrins and derivatives thereof can be used to solubilize the compounds before they are incorporated into liposomes. Cyclodextrins and derivatives thereof can also help to improve the stability of agents in these formulations. This can be particularly important when lack of stability of the agent contained in the liposomes is a concern during prolonged storage *in vivo* or *in vitro*.

Other types of carriers may be readily apparent to those of ordinary skill, and the application of inclusion complexes and cyclodextrin derivatives as described above for such carriers

will be within the scope of this invention.

IV. COMPOSITIONS AND METHODS OF USE

It will be readily apparent that this invention encompasses many different compositions and uses for cyclodextrins, their derivatives and inclusion complexes formed therewith.

Where a cyclodextrin or derivative thereof is to be used as or with a pharmaceutical agent for therapeutic or diagnostic purposes, it may be used in combination with any pharmaceutically accepted excipients and adjuvants, as well as the carriers described above. The compositions may be in any pharmaceutically acceptable form for any type of administration, including topical, oral, rectal or parenteral administration. And because cyclodextrins and derivatives thereof can mask taste, different types of oral administrations may be possible, including chewable or effervescent tablets (e.g., for antacids), gargles, topical solutions and suspensions, pastes, ointments, etc. All forms of such compositions, including those which have been freeze-dried and spray dried are within the scope of this invention. Methods of treating a patient, including a human, will comprise administering therapeutically effective amounts of such compositions. Preparation and administration of such compositions will be within the skill of persons in such arts.

Additionally, because cyclodextrins facilitate the prolonged release of pharmaceutical agent, such compositions, for example, may be administered, with or without carriers, inside of body cavities where the agent would be released more slowly than if it were in the form of the free drug. Examples of where such compositions could be employed include:

(a) the abdomen for localized, prolonged release of pharmaceutical agents, especially antibiotics for infections or antineoplastics for locally treating cancer in the peritoneum or other cavity in this site;

(b) the meningeal space for the treatment of meningeal infection or meningeal cancers;

(c) the pleural or pericardial spaces for the treatment of infection or cancer;

(d) the skin, subcutaneous tissue or muscle;

(e) the vagina for local treatment of cancer or infection, or for slow release of a contraceptive;

(f) the rectum for treating systemic disease in the rectum; and

(g) the organs and glands for treatment of cancer, infection, etc.

Other advantages can also accrue from the aforementioned cyclodextrins, cyclodextrin derivatives and pharmaceutical compositions, including protecting the pharmaceutical agents from enzymes and acids of the gastrointestinal tract, lytic agents in the body such as in the saliva, enzymes in the lungs and in the body, particularly at the surface and within cells such as polymorphic nuclear leukocytes, macrophages and other cells, and possibly also from the destructive mechanisms found within the cytoplasm of most cells.

The cyclodextrin derivatives of this invention may also be used in other ways, for example, to encapsulate proteinase inhibitors. Such complexes may be used therapeutically, e.g., for co-administration with peptides of therapeutic interest. Alternatively, such complexes may be used for separating proteins, e.g., by chromatographic means.

Such compounds and compositions may also protect the patient from allergic reactions to the agent. This may be accomplished if the potential antigenic sites on the agent are included or otherwise shielded such that they are no longer immunogenic, and such that they are protected from attacking antibodies or lymphocytes. In such instances, the complex or prodrug likely would be administered parenterally in order to introduce the cyclodextrin into the bloodstream.

For example, penicillin in some individuals is antigenic, giving rise to anti-penicillin antibodies. In sensitized

individuals, however, penicillin compositions in accordance with this invention may well be "hidden" form the antibody and no such system anaphylaxis will occur. Moreover, if such penicillin compositions were initially administered, sensitivity may be prevented in the first instance. Other substances which are potentially immunogenic include antibodies, peptides (discussed below) or other synthetic or natural materials.

Similarly, such compositions may prevent unwanted binding of an agent to a specific receptor on a cell, thereby preventing unwanted destruction by the cell. This could effectively prolong the half-life of the agent in circulation. It is noted that many agents act by binding to a receptor which then causes some appropriate function, and that hiding this site may decrease the therapeutic potential of the agent. There may be circumstances, however, where it is more desirable to have the agent act at a certain site (e.g. bacteria) and not bind to other cells which carry the receptor.

It will be readily appreciated that many different pharmaceutical agents can be delivered using the cyclodextrins, cyclodextrin derivatives and pharmaceutical compositions described above. Several specific applications, however, merit further discussion.

A. Cimetidine

It has been discovered that the solubility of cimetidine (Tagamet) in water is greatly enhanced in compositions comprising cyclodextrin derivatives in accordance with this invention. This enhanced solubility ameliorates side-effects associated with administration of drug, including bad taste.

Accordingly, another embodiment of this invention comprises an inclusion complex comprising cimetidine included in a cyclodextrin derivative, or a cyclodextrin prodrug comprising the residue of cimetidine covalently bonded to a cyclodextrin derivative as described herein or in the '359 Application, or a

pharmaceutical composition containing such inclusion complex or prodrug. Any of the aforementioned forms of pharmaceutical compositions is envisioned, including those comprising carriers for targeted or prolonged delivery.

Advantageously, the cyclodextrin derivative is of the formula CD - W - R^{13} - L, which is described above. Advantageously, W represents an optional, functional linking group such as amino, amide, ester, thioether, thioamide, thioester, etc.,

R^{13} represents an optional arm such as substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl, and

L represents an optional group selected from reactive, charged, polar or associating groups, e.g., amino, carboxyl, hydroxyl, sulfonate and phosphate.

One preferred group of compounds within the above formula comprises cyclodextrin derivatives in which R^{13} represents alkyl groups of from 1-3, 1-6, and 1-10 carbons. Those of from 10-20 comprise another preferred group, and those of greater than 20 carbons comprises yet another preferred group. Yet another preferred group comprises cyclodextrin derivatives in which L represents a negatively charged group, for example carboxyl, hydroxyl, sulfonate and phosphate. Yet another preferred group comprises cyclodextrin derivatives in which W is amino, amide or ester.

Another preferred group useful in forming inclusion complexes with cimetidine comprises cyclodextrin derivatives having the
formula CD - X - R^{14} - Q, which is described above. Advantageously,

R is as described above, and preferably represents alkyl groups of from 1-3, 1-6, 1-10, 10-20 or greater than 20 carbons; and

Q is a carboxylic acid group.

It has been found, for example, that the stability constant for the inclusion complex formed between 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-ß-cyclodextrin (β-CDNSc) and cimetidine is much higher than that for the analogous complex involving ß-cyclodextrin. Moreover, the solubility of cimetidine in water can be increased to greater than 80 mg/mL. Such complexes have also proven effective in taste masking of such orally-administered solutions of cimetidine.

B. Peptides

The present invention provides a compositions and methods for effectively stabilizing, solubilizing, and/or enhancing the delivery or activity *in vivo* or *in vitro* of polypeptides, especially proteins, by means of the cyclodextrin derivatives described herein and in the '359 Application. Such compositions may be in the form of an aqueous solution. The resulting compositions comprising the polypeptides are also within the scope of this invention. Lyophilized forms of such compositions are also within the scope of this invention. Preferably, the foregoing methods and compositions are directed toward improvements in biologically useful protein formulations.

It has been found that formulations containing a combination of a therapeutic polypeptide and a cyclodextrin or modified cyclodextrin are suitable for oral administration. Partial encapsulation of the polypeptide in the cyclodextrin may prevent the enzyme-drug interaction and hence limit enzymatic degradation of the polypeptide. The rate of degradation of the polypeptide will of course only be reduced when the effective concentration of the polypeptide is below the K_m of the enzyme. However, in vivo, most enzymes work below the K_m so for purposes of drug delivery this condition will in most cases be fulfilled.

Surprisingly, it has been found that cyclodextrins are suitable for the control of treatment of metabolic disorders

associated with compounds produced *in vivo* through enzyme catalysis. For example, where a disorder results from the over-production of a physiologically active compound, and the production of that compound is enzyme-catalyzed, competitive binding of the substrate of the enzyme to a cyclodextrin can limit that over-production. Alternatively, encapsulation of the product of the enzyme-catalyzed reaction may limit the undesirable physiological effects of that compound. Other therapeutic benefit may be derived through maintaining a larger substrate pool by substrate binding to cyclodextrins.

One area where this approach has considerable benefit is in the treatment of metabolic disorders associated with the *in vivo* production of the catecholamine neurotransmitters, noradrenaline and adrenaline, from tyrosine. Cyclodextrins used for this purpose could be administered by injection.

Each of these therapeutic applications involves the inhibition of enzyme activity by competitive binding of enzyme substrates to cyclodextrins and modified cyclodextrins. Example 120 below demonstrates this inhibition in an *in vitro* model system. As demonstrated in that Example, the interaction of α-chymotrypsin with a model substrate, N-benzoyl-L-tyrosine ethyl ester, is reduced by competitive binding of the substrate to β-cyclodextrin. N-Benzoyl(l)-tyrosine ethyl ester (BTEE) is a substrate commonly used to determine the activity of α-chymotrypsin. It is shown that the rate of hydrolysis of BTEE by α-chymotrypsin is slowed in the presence of β-cyclodextrin (β-CD).

The polypeptides intended for use according to the present invention are any polypeptides which are biologically or industrially useful, i.e., one which can be employed in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and conditions in man or animals. Polypeptides, especially proteins, for use in human and/or veterinary medicine or in

diagnosis (*in vivo* or *in vitro*) are of particular interest. Industrially useful polypeptides are those employed analytically, in production or which are otherwise useful in chemical industry.

Amino acids are organic compounds that have at least one amino ($-NH_2$) group and at least one carboxyl (-COOH) group. Oligopeptides are amino acid oligomers which are formed by reacting amino and carboxyl groups from different amino acids to release a water molecule and form a peptide linkage. Polypeptides are amino acid polymers which are formed in the same way. Generally, oligopeptides have between 2 and 20 peptide linkages whereas polypeptides have more. Proteins are very large polypeptides.

The amino acids which make up the polypeptides of the present invention may be natural or synthetic, may have the amino group attached to any non-carbonyl carbon atom (e.g., to the α, β, γ, δ or ϵ carbon as measured from the α-COOH group of the amino acid), may exist as enantiomers (e.g., D- or L-) or diasteriomers, may have blocked carboxyl or amino groups and may have side chains which are hydrophobic, hydrophilic, acidic, basic or neutral. The synthetic polypeptides may contain reversed peptide linkages, in which case they must contain at least one diamine and one dicarboxylic acid monomer.

The polypeptides intended for use in the methods and compositions of this invention include molecules to which nonpeptide prosthetic groups, such as carbohydrates, hemes and fatty acids, have been attached. The polypeptides include molecules made by living organisms or cells, molecules made by synthetic organic chemistry and molecules which are synthetically modified biological products. They may have an amino acid sequence identical to that of a natural substance or one altered by techniques such as site-directed mutagenesis.

In addition to the covalent (primary) structure, the polypeptides may possess unique conformations (combinations of

secondary, tertiary and quaternary structure) which affect their biological function, aqueous solubility and ability to interact with the cyclodextrins. The polypeptides may have many biological functions. They may act as enzymes; enzyme inhibitors, antibodies; antigens; transporters of electrons, oxygen, metal ions, or small organic molecules; ionophores; antibiotics; mitogens; hormones; growth regulators; neurotransmitters; cell surface recognition proteins; cell chemotactic factors; and cytotoxins. They may also be receptors, agonists, antagonists of the following: ionophores, antibiotics, mitogens, hormones, neurotransmitters, growth regulators, cell surface recognition proteins, cell chemotactic factors and cytotoxins.

Uses for some of the preferred polypeptides of the invention include: immunization (as vaccine adjuvants), *in vitro* diagnostics (to increase the solubility/stability or lower the nonspecific binding of antigens or antibodies) and *in vivo* diagnostics and therapeutics (to increase the solubility and/or stability of therapeutic and diagnostic polypeptides). Preferred therapeutic targets of these polypeptides are cancers, such as melanoma, renal cell carcinoma, myeloma, leukemia, breast cancer, colorectal cancer, lymphoma, neuroblastoma, astrocytoma and glioma; auto-immune diseases, such as diabetes mellitus, rheumatoid arthritis and multiple sclerosis; immunodeficiency diseases; and infectious diseases.

Among the polypeptides contemplated by the present invention are therapeutically useful polypeptides such as anti-sera, anti-toxins and antigens. Anti-sera may include, for example, antirabies, antivenin (black widow spider venom), hepatitis B immune globulin, tetanus immune globulin, intravenous immune globulin, pertussis immune globulin and rabies immune globulin. Anti-toxins may include, for example, those for diphtheria and tetanus; Rho(D) immune globulin; serum components, such as 5% normal human serum albumin, 5% plasma protein fraction, 20% normal human serum albumin, 25% normal human serum albumin.

Also useful are factor II, factor VII, factor VIII, factor IX, factor X and Xa, antithrombin III, transferrin, haptoglobin, fibronectin, gamma globulins, protein C, protein S and thrombin; toxoids, such as diphtheria and tetanus; vaccines, including attenuated vaccines (such as those for cholera, influenza, meningitis, *Yersinia pestis* or plague, pneumonia, poliomyelitis, rabies, typhoid and staphylococcus) and live vaccines (such as those for poliomyelitis, measles, rubella and mumps); growth factors, hormones and like bioactive peptides, as illustrated by α-1-antitrypsin, atrial natriuretic factor (diuretic), calcitonin, calmodulin, choriogonadotropin (α and β), colony stimulating factor, corticotropin releasing factor, β-endorphin, endothelial cell growth supplement, epidermal growth factor, erythropoietin, fibroblast growth factor, fibronectin, follicle stimulating hormone, granulocyte colony stimulating factor, growth hormone (somatotropin), growth hormone releasing factor (somatoliberin).

Also useful are insulin, insulin-like growth factor, (somatomedin), an interferon (typically α, β, γ), an interleukin (typically 1, 2, 3, 4), lutropin, lymphotoxin, macrophage derived growth factor, macrophage inhibiting factor, macrophage stimulating factor, megakaryocyte stimulating factor, nerve growth factor, pancreatic endorphin, parathyroid hormone, platelet derived growth factor, relaxin, secretin, skeletal growth factor, superoxide dismutase, thymic hormone factor, thymic factor, thymopoeitin, thyrotropin, tissue plasminogen activator, transforming growth factor (α and β), tumor necrosis factor, tumor angiogenesis factor, vasoactive intestinal polypeptide and wound angiogenesis factor; immunosuppressives, such as RhO (D) ISG and IVGG's, thrombolytics such as urokinase, streptokinase and tissue plasminogen activator; and antigens such as Rhus all (poison ivy), Rhus tox poison ivy-polyvalent and staphage lysate (staphylococcus lysate).

Other polypeptides contemplated by this invention are polypeptides specifically intended to veterinary use, including

vaccines, animal growth factors and bovine interferons and interleukin-2.

Illustrative vaccines include: bovine vaccines, for example those for anthrax, clostridium (multiple species), pasteurella, leptospira pomona, bovine diarrhea, brucillosis, parainfluenza, 3-respiratory syncytial virus, tetanus, vesicular stomatitis and staphylococcus; canine vaccines, for example those for bordetella, coronavirus, distemper, parvovirus, parainfluenza and rabies; equine vaccines, for example those for anthrax, encephalomyelitis, influenza, tetanus, rabies and streptococcus-strangles; feline vaccines, such as those for leukemia, pneumonitis-chlamydia and rabies; ovine vaccines, for example those for anthrax, blackleg, bluetongue, enterotoxemia, tetanus and vibriosis; and porcine vaccines, for example those for anthrax, enterotoxemia, dysentery, erysipelas, leptospirosis, parvovirus, pseudorabies, tetanus and rotavirus.

Yet other polypeptides contemplated by this invention have use in immunology. These include monoclonal antibodies, polyclonal antibodies (unconjugated), second antibodies (alkaline phosphatase conjugated), immunoglobulin screening and isotyping kits, protein A products and immunoassay reagents. Among the monoclonal antibodies are those approved for use in diagnostic kits, for example IgE, peroxidase-anti-peroxidase conjugated, human chorionic gonadotropin, T cell, ferritin conjugated, carcinogenic embryonic antigen (CEA), OKT-II, anti-rabies, human growth hormone, Total T4, prolactin, 1251-IgE, UCG, thyroid stimulating hormone, chlamydia, gentamicin and rubella. Other useful monoclonal antibodies include monoclonal antibodies for human cell surface antigens, monoclonal antibodies for murine cell surface antigens, monoclonal antibodies to complement and blood proteins, monoclonal antibodies to immunoglobulins (human), monoclonal antibodies to neurological antigens, monoclonal antibodies to tumor markers, monoclonal antibodies to cell components, Epstein Barr virus antigens, human lymphocyte antigen (HLA) typing, hematology antibodies, leucocyte antibodies,

bacterial antigens, parasitic antigens, T-cell lymphotropic virus (HIV-III) and cytoskeletal antibodies. Useful polyclonal antibodies (unconjugated) include affinity purified antibodies to immunoglobulins, antibodies to plant viruses, antisera to human isoenzymes and chromatographically purified antibodies. Useful alkaline phosphatase conjugated second antibodies include affinity purified antibodies to immunoglobulins, antibodies to plant viruses, biotin-conjugated antibodies, fluorescein-isothiocyanate-conjugated antibodies (FITC), gold-conjugated antibodies, peroxidase-conjugated antibodies, rhodamine conjugated antibodies and iodine-conjugated antibodies. Polypeptide immunoassay reagents include EIA grade enzymes, enzyme-antibody complexes, reagents for immunology, enzyme linked immunosorbent assays (ELISA) for use as standards or controls, immunoelectrophoresis (IEP) assays, radioimmunoassays (RIA) for use as standards or controls, nephelometry for use as standards or controls, nuclear antigens and coatings for kit tubes and plate wells.

Other polypeptides contemplated by the present invention include polypeptides useful in cell biology/biochemistry, for example in serum-free cultures (as supplements and reagents for cell culture), in glycoprotein and carbohydrate research (endoglycosidoses, exoglycosidoses, enzymes for carbohydrate research, enzymes for analysis of glycoprotein oligosaccharides), as molecular weight markers (calibration proteins, e.g., for gel permeation chromatography, subunit proteins), proteases (for use in blood research, protein sequencing, tissue digestion and cell harvest, total digestion of proteins and immobilized proteases), cell surface recognition proteins (adenosine and analogs, cyclic nucleotides, phosphoinositides), phospholipases and bioluminescence assay reagents.

Yet other polypeptides contemplated for use herein are polypetides of particular interest in the field of molecular biology, including various enzymes and reagents. The enzymes can include labelling enzymes, modifying enzymes, nucleases,

polymerases, sequencing enzymes and restriction enzymes. The reagents can include inhibitors, antibiotics and miscellaneous other reagents.

Preferred polypeptides for use in accord with the instant invention include growth regulators. Among the preferred growth regulators are hematopoietic factors, which affect the maturation and proliferation of blood cells in lymphoid tissue and bone marrow; cytokines, which generally influence eukaryotic cell growth; and lymphokines, which affect lymphocyte growth. Specific polypeptides which are growth regulators or lymphokines are: interleukin 1, 2, 3 and 4; α, β, and γ interferons; granulocyte colony stimulating factor (G-CSF); granulocyte - macrophage CSF (GM-CSF); macrophage CSF(m-CSF); megakaryocyte CSF; multi CSF or IL-3 (also known as BPA, HCGF, MCGF and PSF); erythropoietin; lymphotoxin; tumor necrosis factor (TNF, also known as cachectin); α and β transforming growth factor (TGF); platelet derived growth factor (PDGF); epidermal growth factor (EGF); nerve growth factor (NGF); insulin-like growth factor I and II (IGF I is also called somatomedin C); growth hormone (GF, also called somatotropin); and growth hormone releasing factor (GHRF, also called somatoliberin). See Clark et al., "The Human Hematopoietic Colony Stimulating Factors," *Science*, 1229-1237 (June 5, 1987); Taniguchi, "Regulation of Cytokine Expression," *Ann. Rev. Imm.*, 6, 439-464 (1988); and Watson et al., *Molecular Biology of the Gene*, Vol. II, 4th Ed., Benjamin/Cummings Publishing (1987).

Other preferred polypeptides for use in accordance with this invention are fusion proteins. Fusion proteins are covalently linked proteins or portions of proteins. Thus, proteins or active fragments thereof having different purposes can be linked to provide a fused molecule having characteristics of both. Generally speaking, one of the proteins/protein fragments is a "seeker" and the other an "actor" or "destroyer".

Illustrative such fusion proteins are pseudomonas exotoxin

linked to IL-2, diphtheria toxin linked to IL-2 or either toxin linked to other proteins, or linkages between other proteins such as those preferred proteins described in the preceding paragraph, or portions thereof. See United States Patent Nos. 4,545,985; 4,468,382; and 4,675,382; all of which are incorporated by reference herein in their entireties and relied upon.

Fusion proteins of IL-2 linked with toxin as described above are designed to kill cells with IL-2 receptors, thus find use in preventing graft-versus-host rejection. Other fusion proteins have varying utilities, depending on the proteins or portions thereof which are combined. Thus, for example, Substance P and a toxin form a fusion protein which can be used to relieve chronic pain, while α-melanocyte-stimulating hormone (MSH) and diphtheria toxin form a fusion protein designed to kill melanoma cells.

Yet other preferred polypeptides for use herein are muteins, which are mutationally altered proteins. Preferred muteins are muteins of the preferred growth regulators and fusion proteins identified in the preceding paragraphs, and typically have the same purpose as the corresponding unaltered proteins. Especially preferred muteins include IL-2 muteins, described for example in United States Patent Nos. 4,752,585 and 4,518,584, incorporated by reference herein in their entireties and relied upon; and muteins of β-interferon, described for example in United States Patent Nos. 4,737,462 and 4,588,585, incorporated by reference herein in their entireties and relied upon.

It will be appreciated that the aforementioned polypeptides may be used in the form of inclusion complexes with any of the cyclodextrin derivatives described herein in the '359 Application, or may be covalently bound to any such cyclodextrin derivative such that the covalent bond, when broken, will yield the polypeptide in useful form, e.g., a prodrug.

C. **Amiodarone**

As discussed above, advantageous compositions comprising amiodarone have been formulated for both oral and intravenous administration. Using a 1:4 (amiodarone: β-CDNSc) molar ratio, the solubility of amiodarone in aqueous solution was increased 35 times. As seen from Examples 137 and 138 below, such compositions provided oral and intravenous delivery profiles which were improved in several respects.

Accordingly, another embodiment of this invention comprises an inclusion complex comprising amiodarone included in a cyclodextrin derivative, or a cyclodextrin prodrug comprising the residue of amiodarone covalently bonded to a cyclodextrin derivative as described herein or in the '359 Application, or a pharmaceutical composition containing such inclusion complex or prodrug. Any of the aforementioned forms of pharmaceutical compositions is envisioned, including those comprising carriers for targeted or prolonged delivery. Advantageously, the cyclodextrin derivative is of the formula CD - W - R^{13} - L, or CD - X - R^{14} - Q, which are described above, including preferred groups.

D. **Piroxicam**

Another embodiment of this invention provides inclusion complexes and pharmaceutical compositions Piroxicam and related non-steroidal anti-inflammatory drugs with cyclodextrin derivatives as described herein and in the '359 Application. Such compositions may improve the therapeutic performance of Piroxicam and related non-steroidal anti-inflammatory drugs, for example, through increases in their rates of dissolution and their solubilities as a consequence of their formulation with such cyclodextrin derivatives. These inclusion complexes and pharmaceutical compositions may also substantially reduce the irritation of the gastro-intestinal tract associated with the oral administration of Piroxicam and related non-steroidal anti-inflammatory drugs. Bioavailability may also be improved.

Embodiments include compositions in which Piroxicam is included in a cyclodextrin derivative (e.g., β-amino cyclodextrin) using a 1:1, 1:2 or 1:4 molar ratio of Piroxicam to cyclodextrin derivative. Another embodiment comprises Piroxicam included in a linked cyclodextrin derivative using a 1:1 mole ratio of Piroxicam to cyclodextrin derivative. In each of the embodiments there is formed a solid inclusion complex having a high wettability and solubility, and dissolves very rapidly to produce both the inclusion complex and free Piroxicam in solution. Other embodiments will be readily apparent. Examples 121, 122 and 129 below illustrate such formulations.

D. Other Pharmaceutical Agents

Inclusion complexes, prodrugs and pharmaceutical compositions comprising many other pharmaceutical agents are also within the scope of this invention. Such agents include Ketoprofen, lorazepam, propranolol hydrochloride, amikacin, nadolol, etoposide, captopril, iopamidol, fosinopril, cefuroxime sodium, beclomethasone, labetalol hydrochloride, ranitidine, ceftazidime, cefuroxime, cefaclor, dobutamine, insulin, fluoxetine, nizatidine, diltiazem, terfenadine, nifedipine, glipizide labetalol, clotrimazole, betamethasone, flutamide, mometasone furoate, auranofin, cefonicid sodium, cimetidine, mupirocin, fenoldopam, amoxillin, clavulanic acid, nabumetone, ticarcillin disodium, ceftizoxime, carvedilol, oxfendazole, naproxen, aspirin, alprazolam, flubiprofen, glyburide, minoxidil, gemfibrozil, acyclovir, naficillin, ampicillin, cephalexin, cephradine, carbidopa, ivermectin, timolol, norfloxacin, tocainide, cefoxitin, lovastatin, famotidine, enalalpril, enalaprilat, salmon calcitonin, human calcitonin, melphalan, chlorambucil, piromidic acid, biphenylylacetic acid, amphotericin B, β-lactam antibiotics, prostaglandins, and non-steroidal anti-inflammatory drugs such as tolmetin, indomethacin, fenoprofen, piroxicam, pirprofen, Ibuprofen, flurbiprofen, mefenamic acid, sulindac and diflunisal, bendrofluazide, furosemide, diclofenac, cardizem, tamoxifen, sulfamethoxazole, amoxicillin, cinnarizine, vinpocetine, bencyclane, tiamulin and carboplatin, propanolol,

chlorobutanol, hydrocortisone, dicumarol, tiamulin, prednisolone, and testosterone.

E. Cyclodextrins as Therapeutic Agents

Surprisingly, it has been found that cyclodextrins are suitable for the control of treatment of metabolic disorders associated with compounds produced *in vivo* through enzyme catalysis. For example, where a disorder results from the over-production of a physiologically active compound (e.g., epilepsy), and the production of that compound is enzyme-catalyzed, competitive binding of the substrate of the enzyme to a cyclodextrin can limit that over-production. Alternatively, encapsulation of the product of the enzyme-catalyzed reaction may limit the undesirable physiological effects of that compound. Other therapeutic benefit may be derived through maintaining a larger substrate pool by substrate binding to cyclodextrins.

One area where this approach has considerable benefit is in the treatment of metabolic disorders associated with the *in vivo* production of the catecholamine neurotransmitters, noradrenaline and adrenaline, from tyrosine. Cyclodextrins and derivatives thereof, including those described herein and elsewhere, used for this purpose could be administered by injection.

Because cyclodextrins may encapsulate free drug, it is possible to intravenously administer cyclodextrins and derivatives thereof in accordance with this invention and the '359 Application intravenously to encapsulate or "mop up" drugs or toxins in the circulation and to release them at a slower rate. For example, it might be determined that the antineoplastic drug Melphalan, which is known to be encapsulated by cyclodextrins and derivatives thereof, may be in too high a concentration in a patient. If cyclodextrins or derivatives thereof were given in large amounts intravenously, they could mop up excess Melphalan, free Melphalan in the circulation and potentially decrease the circulating amount of free material. This could lead to a step wherein then cyclodextrins or

derivatives thereof could be removed from the circulation.

V. SYNTHETIC PROCEDURES AND INTERMEDIATES

This section provides synthetic procedures for preparing various cyclodextrin derivatives and intermediates which can be used in accordance with this invention. For a detailed discussion of additional synthetic procedures and intermediates which may also be used, the reader is invited to review the '359 Application, and particularly Section IV. entitled "Synthetic Procedures and Intermediates" and the references cited therein.

A. Preparation of CD-W-R-L Compounds

As discussed above, one particularly useful group of cyclodextrin derivatives is of the formula CD-W-R-L, wherein

CD represents an otherwise substituted or unsubstituted cyclodextrin,

W represents an optional, functional linking group such as amino, amide, ester, thioether, thioamide, thioester, etc.,

R is as described above for R^{13} and represents an optional arm such as substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl, and

L represents an optional group selected from reactive, charged, polar or associating groups, e.g., amino, carboxyl, hydroxyl, sulfonate and phosphate.

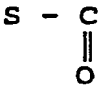
One preferred group of compounds within the above formula comprises cyclodextrin derivatives in which R represents alkyl groups of from 1-3, 1-6, and 1-10 carbons. Those of from 10-20 comprise another preferred group, and those of greater than 20 carbons comprises yet another preferred group. Yet another preferred group comprises cyclodextrin derivatives in which L represents a negatively charged group, for example carboxyl, hydroxyl, sulfonate and phosphate. Yet another preferred group comprises cyclodextrin derivatives in which W is amino, amide or ester.

Compounds of the formula CD-W-R-L have several uses. They can be used as hosts for advantageous inclusion complexes, such as those described above for amiodarone. They may also be used as intermediates for preparing other cyclodextrin derivatives including those in which a useful agent is covalently bound to the cyclodextrin such that the bond, when broken, will yield the agent in an active form. Generally, in such cases L will be a functional group such as amino, carboxyl or a sulfur-containing group.

Several methods for making compounds of the general formula CD-W-R-L, as defined above, are disclosed in the '359 Application, and particularly subsections D-H of Section IV. Those subsections disclose processes for preparing pendant arm cyclodextrins, including those linked by amines (subsection E), sulfur (subsection F), ester (subsection G), and amide (subsection H).

B. Preparation of CD-X-R-Q Compounds

Also as discussed above, a particularly useful group of compounds within the CD-W-R-L genus are those having the formula CD-X-R-Q, wherein:

X represents  or 

R is as described above for R^{14}, and advantageously represents a group such as substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl, and

Q is a carboxylic acid group or a carboxylic acid group derivatized to undergo substitution, e.g., acid chloride, acid anhydride or ester.

Where Q is a carboxylic acid group, such compounds are particularly useful as hosts for inclusion complexes (e.g., with amiodarone). Where Q is a carboxylic acid group derivatized to undergo substitution, e.g., acid chloride, acid anhydride or ester, then such compounds are highly reactive intermediates

useful for preparing other pendant arm intermediates, and cyclodextrin derivatives in which a useful agent is covalently bound to the cyclodextrin such that the bond, when broken, will yield the agent in an active form (e.g., prodrugs). More so, however, this intermediate is extremely useful for preparing asymmetric linked cyclodextrins in which the cyclodextrins are different (α, β, γ) and/or differently substituted, e.g., primary versus secondary. In such instances, the intermediate can then be reacted with a different amino- or thiol-substituted cyclodextrin to yield the asymmetrical linked cyclodextrins. This is particularly useful where it is desired to link a primary carbon of one cyclodextrin to a secondary carbon of another cyclodextrin.

When Q is a derivatized carboxylic acid, the intermediate is highly reactive and should be either used directly or freeze dried and store crystalline in cold. Alternatively, the intermediate can be hydrolyzed to the free acid which is stable indefinitely, and which can be reactivated if desired.

Compounds of the formula CD-X-R-Q can be prepared by reacting an amino cyclodextrin (CD-NH_2) or a cyclodextrin thiol (CD-SH) with a dicarboxylic acid that has been derivatized to undergo substitution. To an extent, this process parallels the reaction for preparing linked cyclodextrins described in scheme 5 in subsection IV.J. of the '359 Application entitled "Preparation of Linked Cyclodextrins." It has been discovered, however, that by controlling the process parameters such as time and the molar ratio of reactants, the production of the desired product, i.e., the derivative CD-X-R-Q, or the linked cyclodextrins can be optimized. (See also subsection IV.H. of the '359 Application entitled "Preparation of Cyclodextrins Substituted With Pendant arms Linked via an Amide" for examples of carboxylic acids derivatized to undergo substitution.)

For example, using the m-nitrophenyl diester of succinic acid as the dicarboxylic acid derivatized to undergo

substitution, and 6^A-amino-6^A-deoxy-β-cyclodextrin as the amino-substituted cyclodextrin, the reaction can be controlled such that the major products are either the succinate-linked cyclodextrins or the m-nitrophenyl ester of 6^A-amino-6^A-deoxy-6^A-N(3-carboxypropanoyl)-β-cyclodextrin. In general, the linked product will be predominant when the molar ratio of amino cyclodextrin to derivatized dicarboxylic acid is at least about 2:1, and the reaction is allowed to proceed for several days at room temperature. If however, the molar ratio is about 1:1 or less, or the reaction is allowed to proceed for only a few hours, the predominant product will likely be the non-linked derivative. Guidelines for preparing and optimizing yields of the linked and non-linked products are provided in Examples 58-65 below. Of course, a skilled artisan may also vary other parameters such as reaction temperature, pressure, etc., and such modifications are well within the scope of this invention.

Alternatively, the free acid form, i.e., where Q is the carboxylic acid, can be directly obtained, for example, by using as the derivatized dicarboxylic acid reactant an anhydride such as succinic anhydride. This intermediate form is stable indefinitely, and can subsequently be activated, e.g., by making a p-nitrophenyl ester, to undergo substitution.

C. Preparation of Linked Compounds

Thus, provided herein and in the '359 Application are several preferred methods for preparing linked compounds. *Inter alia*, where it is desired that the cyclodextrins be linked by ester-containing linking groups, or where a symmetrical linked cyclodextrin is desired, then the process described in scheme 4 in subsection IV.J. of the '359 Application entitled "Preparation of Linked Cyclodextrins" can be used. Where, amide or thioamide linkages are desired, or where an asymmetrical linked cyclodextrin is desired, the reactions described above can be employed. It has been found that in order to obtain high yields of the linked cyclodextrins, amine derivatives should be purified to a high degree before the next step in the synthesis.

Purification of the linked product may be accomplished using Sephadex.

It has been discovered that the reaction rate for forming linked cyclodextrins also depends upon the cyclodextrin annulus size and the length of the linking group. This is believed to be due, at least in part, to the ability of the linking group to "fold" back and include in the cyclodextrin cavity, thereby obscuring the reactive group. Thus, it is believed that shorter linking groups will be less likely to fold back and include back into the annulus and obscure the reactive group. This correlates with the observations that cyclodextrins linked by oxalate have a faster reaction rate than if linked by will malonate, which is in turn faster than if linked by succinate, etc.

Similarly, it is believed that the same general phenomenon accounts for longer reaction times for linking β-cyclodextrins compared with α-cyclodextrins. The larger annulus of the β-cyclodextrin permits more inclusion than the smaller α-cyclodextrin annulus. Therefore, assuming everything else equal, α-cyclodextrins will produce linked species faster than β-cyclodextrins, etc.

D. Preparation of Secondary Amino-Deoxy-Cyclodextrins

Several possible synthetic routes for preparing secondary amino-deoxy-cyclodextrins are provided below. For purposes of illustration, the syntheses will be discussed using secondary m-nitrobenzensulfinyl-α-cyclodextrin as a key intermediate. However, those skilled in the art will appreciate that cyclodextrins substituted with other leaving groups such as tosylates and iodides can be similarly used as a satisfactory intermediate. The choice of preferred intermediate may well be dictated by the ease of its preparation and purification. For example, Fujita et al. J. Am. Chem. Soc. 1985, 107, 3233 prepares 2^A-O-m-nitrobenzenesulfinyl-α-cyclodextrin in good yield and also prepares (J. Am. Chem. Soc. 1986, 108, 2030) 3^A-O-β-naphthalenesulfinyl-β-cyclodextrin in good yield. A preferred

reagent can be found to substitute, with a leaving group, the cyclodextrin of choice at the carbon of choice, to prepare in optimal yield a suitably cyclodextrin intermediate. This intermediate can be purified by the surprisingly highly efficient reverse phase chromatography procedure disclosed in the '359 Application.

1. Preparation of a Secondary Amino-Deoxy-Cyclodextrins From C2 Substituted Cyclodextrin Intermediates

Cyclodextrin manno epoxides can be prepared as described by the method of Breslow et al. J. Am. Chem. Soc. 1983, 105, 1390. For example, 2^A-m-nitrobenzenesufinyl-α-cyclodextrin is treated with base and heated to give α-cyclodextrin manno epoxide in good yield. Best results are obtained if the starting materials are pure. Purification of the epoxide is easily effected by Sephadex^R chromatography. Subsequent treatment of the manno epoxide with ammonium hydroxide or ammonia leads to a 3^A-amino-3^A-deoxy-cyclodextrin where the stereochemistry at both C2 and C3 of the substituted glucose has been inverted from the normal cyclodextrin stereochemistry. Purification of the product using standard techniques, for example, Sephadex^R chromatography is effective.

The cyclodextrin manno epoxide need not be isolated. Treatment of 2^A-m-nitrobenzenesulfinyl-α-cyclodextrin with ammonium hydroxide effects both the formation of the manno epoxide and the subsequent formation of the inverted 3^A-amino-3^A-deoxy-cyclodextrin. Treatment of 2^A-m-nitrobenzenesulfinyl-cyclodextrins with azide ion (for example, sodium azide in water) in a manner similar to that described in the preparation of 6^A-azido-6^A-deoxy-cyclodextrin in the '359 Application will provide 2^A-azido-2^A-deoxy-cyclodextrins in good yield. Reduction of the azide with, for example, hydrogen over palladium will yield 2^A-amino-2^A-deoxy cyclodextrins. In this case, the stereochemistry of the substituted glucose remains unchanged.

2. Preparation of Secondary Amino-deoxy-Cyclodextrins from C3 Substituted Cyclodextrin Intermediates

In this synthesis, 3^A-O-β-naphthalenesulfinyl-β-cyclodextrin can be treated with base and heated to give β-cyclodextrin allo epoxide. This procedure is similar to that described above for preparing the manno epoxide. Conditions and methods of purification of the manno epoxide. Conditions and methods of purification are also similar. Treatment of the epoxide with ammonium hydroxide or with ammonia leads to a 2^A-amino-2^A-deoxy-cyclodextrin where the stereochemistry at both C2 and C3 of the substituted glucose has been inverted from the normal cyclodextrin stereochemistry. Purification using Sephadex^R chromatography will prove effective. Again, the cyclodextrin allo epoxide need not be isolated. Treatment of 3^A-β-naphthalenesulfinyl-β-cyclodextrin with ammonium hydroxide effects both the formation of the epoxide and the subsequent formation of the inverted 2^A-amino-2^A-deoxy-cyclodextrin.

The following Examples are provided only to illustrate various embodiments of this invention, and do not in any manner of pilot plant scale quantities of cyclodextrin derivatives are incorporated herein by reference.

The procedures, equipment and instruments used in Examples 10 to 74 were as follows.

Thin layer chromatography (t.l.c.) was performed using Kieselgel 60 F_{254} (Merck) on aluminium backing plates. Running solvents used were: solvent A, 14:3:3 butanone-methanol-water; solvent B, 1:8:1 chloroformacetic acid-water; solvent C, 14:3:3 ethyl acetate-methanol-water. Visualization was achieved by dipping the plate in either a solution of diphenylamine (0.1 g), aniline (0.5 ml) and 85% phosphoric acid (1 ml) in acetone (10 ml), or a solution made up of 10:1 acetone - 15% sulfuric acid; and heating to char the spots.

High Performance Liquid Chromatography (HPLC) was carried

out with a Waters Model 510 solvent delivery system coupled to a Waters Model 410 differential refractometer. Unless otherwise specified the column used is a Waters 3.9 x 300 mm Carbohydrate Analysis column and was run at 1.5 $ml.min^{-1}$. Infra-red spectra were recorded on a Jasco A102 grating spectrophotometer, a Hitachi 270-30 spectrophotometer or a Perkin-Elmer Model 1720 Fourier Transform spectrophotometer. Samples were prepared as either their Nujol mulls between sodium chloride plates, or as part of a KBr disc. Mass spectra were recorded on a VG ZAB 2HF mass spectrometer using the FAB technique with Xe or Ar as the collision gas. The samples were dissolved in H_2O or DMSO and introduced into the spectrometer in a glycerol matrix. For some samples it was necessary to add a small quantity of trifluoroacetic acid (TFA) to produce an adequate spectrum. ^1H N.m.r. spectra were recorded on a Varian T60 or Bruker CXP300 spectrometer and ^{13}C n.m.r. were recorded on a Bruker CXP300 or WP80 spectrometer. Solvents and internal standards are mentioned individually within the text. All ^1H n.m.r. samples were dried overnight *in vacuo* over P_2O_5. Melting points were determined using a Reichart melting point apparatus and are uncorrected.

Where multi-O-*p*-toluenesulfonyl substituted cyclodextrin derivatives are mentioned in these examples it should be noted that the exact number and position of substituted hydroxyls is unknown, unless otherwise stated.

Pyridine was dried by storing over freshly activated 4Å molecular sieves. *p*-Toluenesulfonyl chloride was purified according to Vogel, Textbook of Organic Chemistry, 4th Ed., p. 317 and stored in a vacuum desiccator over phosphorus pentoxide. α-Cyclodextrin (α-CD) was supplied by Sigma Chemical Co. or Nihon Shokuhin Kako Co. and contained 3% water. β-Cyclodextrin (β-CD) was supplied by Nihon Shokuhin Kako Co. and contained 10% water. β-CD was stored *in vacuo* over phosphorus pentoxide to constant weight before use. It may also be preferred to dry α-CD before use in the same manner. Other cyclodextrin derivatives, if required in an anhydrous form should be dried similarly.

EXAMPLE 10

6^A-O-*p*-Toluenesulfonyl-α-cyclodextrin (α-CDOTs)

α-CD (8.0 g) was dissolved in pyridine (800 ml) by gentle warming and shaking. *p*-Toluenesulfonyl chloride (8.0 g) was added in one portion and the solution stirred at room temperature for 2 hours. The solution was poured onto a mixture of ice cold 6:1 acetone - ether (6 l) and a fine white precipitate formed which was allowed to settle over 1 hour. Most of the supernatant was decanted and the solid collected by gravity filtration (Whatman № 1 filter paper). The filter cake was washed with cold acetone (100 ml in portions) and allowed to air dry overnight to give 6.6 g of crude product. T.l.c. (solvent A) of the crude product showed: R_C (relative to α-CD), α-CD, 1; α-CDOTs, 1.5; α-CD$(OTs)_2$, 1.75. HPLC of the crude product using a 70% CH_3CN-H_2O eluant showed: t_R (Relative to α-CD), α-CD$(OTs)_2$, 0.38; α-CDOTs, 0.52; α-CD, 1.

The crude product (6.6 g) was dissolved in 30% aqueous methanol (100 ml), filtered and loaded via the pump onto a 19 x 150 mm C_{18} μ-Bondapack HPLC column. This was eluted with 30% aqueous methanol at 15 $ml.min^{-1}$ and gave pure fractions of α-CD (0 - 35 minutes) and α-CDOTs (1.83 g, 45 - 120 minutes). This separation procedure is unexpectedly very effective for up to 8 g quantities of the crude product. The column was washed with several column volumes of methanol to elute multi-O-*p*-toluenesulfonyl substituted cyclodextrins.

FAB MS $M+H^+$ requires 1127, found 1127; $M+Na^+$ requires 1149, found 1149. ^1H n.m.r. (D_6DMSO, $CDCl_3$; TMS standard) δ_H 2.45, CH_3; 3.2 - 5.7, 59H, 7.4 - 7.8, C_6H_4. ^{13}C n.m.r. (D_6DMSO) δ_C 25.2_5, 64.0_3, 73.0_6, 73.7_3, 75.7_2, 76.1_7, 77.1_5, 77.3_3, 85.6_1, 86.1_5, 105.6_1, 106.0_5, 131.7_4, 134.0_1, 136.5_4, 148.9_0.

EXAMPLE 11

6^A-Azido-6^A-deoxy-α-cyclodextrin (α-CDN$_3$)

α-CDOTs (540 mg) and sodium azide (540 mg) were dissolved in water (54 ml) and the solution heated on a boiling water bath for 90 minutes. The solution was concentrated *in vacuo* to approximately 1.5 ml and 1,1,2,2-tetrachloroethane (TCE, 0.25 ml) was added. After vigorous shaking the mixture was left to stand in ice for 10 minutes. The resulting precipitate was collected by centrifugation (3000 rpm, 5 minutes) and washed with ice cold water (2 x 1.5 ml). The precipitate was resuspended in water (54 ml) and heated on a boiling water bath to release TCE which was removed by pipette. The solution was dried *in vacuo* to give α-CDN$_3$ (410 mg). T.l.c. (solvent A) showed: R_C (relative to α-CD), α-CDN$_3$, 0.72. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-CDN$_3$, 0.64.

FAB MS M+H$^+$ requires 998, found 998; M+Na$^+$ requires 1020, found 1020. ν_{max} 2104 cm^{-1}. ^{1}H n.m.r. (D_6DMSO, $CDCl_3$; TMS standard) δ_H 3.2 - 5.7, 59H. ^{13}C n.m.r. (D_6DMSO, $CDCl_3$, TMS standard) δ_C 53.0, 60.4$_3$, 70.5$_8$, 72.1$_4$, 72.3$_3$, 73.4$_0$, 82.1$_8$, 83.0$_8$, 102.1$_0$.

Examples 12 and 13 illustrate alternative syntheses for preparing 6^A-amino-6^A-deoxy-α-cyclodextrin.

EXAMPLE 12

6^A-Amino-6^A-deoxy-α-cyclodextrin (α-CDNH$_2$)

α-CDN$_3$ (3 g) was dissolved in water (90 ml) and palladium black (90 mg) was added. The mixture was shaken on a Parr hydrogenator under hydrogen (30 psi) at room temperature overnight. After venting the hydrogen, TCE (0.1 ml) was added to the mixture which was then shaken until an emulsion was obtained. After standing for 1 hour the solution was filtered (Whatman № 1 filter paper) and the clear colorless solution was evaporated to dryness to give α-CDNH$_2$ (2.84 g). T.l.c. (solvent A) showed: R_C (relative to α-CD), α-CDNH$_2$, 0.3. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-CDNH$_2$, 1.2.

FAB MS $M+H^+$ requires 972, found 972; $M+Na^+$ requires 994, found 994. ^1H n.m.r. (D_2O) δ_H 3.0 - 5.2, 42H. ^{13}C n.m.r. (D_2O; dioxane standard) δ_C 42.8_0, t, 61.7_1, t, 73.2_1, d, 73.7_5, 74.7_3, d, 82.5_5, d, 84.3_7, d, 102.8_0, d.

EXAMPLE 13

α-CDOTs (1.0 g) was dissolved in dry N,N-dimethylformamide (DMF, 50 ml) in a 400 ml pressure vessel. Condensed ammonia (100 ml) was added carefully and the vessel sealed. The pressure increased to 150 psi as the reaction mixture warmed from -33°C to 22°C (room temperature). The reaction mixture was stirred at room temperature for 3 days. The ammonia was vented off and the DMF solution was dried *in vacuo*. The residue was dissolved in water (10 ml) and evaporated *in vacuo* to dryness twice to remove any residual DMF. The residue was dissolved in 20% aqueous ammonia (10 ml) and dropped into acetone (200 ml). The precipitate was collected by vacuum filtration and rinsed with acetone (5 ml) and ether (5 ml) to give 670 mg of white powder. T.l.c. and HPLC showed a spot and peak corresponding to α-$CDNH_2$.

EXAMPLE 14

6^-O-*p*-Toluenesulfonyl-β-cyclodextrin (β-CDOTs)

β-CD (13 g) was dissolved in pyridine (100 ml). *p*-Toluenesulfonyl chloride (1.7 g) was added over a period of 45 minutes with stirring, and the clear solution allowed to stand at room temperature overnight. The pyridine was removed *in vacuo* and the resulting oily residue triturated with acetone (100 ml). The solid residue was separated by filtration and dissolved in boiling water (50 ml). After cooling, the precipitate was separated by filtration, redissolved in the minimum amount of hot water (50 ml) and filtered hot to remove the less soluble β-$CD(OTs)_2$. The filtrate was cooled and the solid collected and dried *in vacuo* over phosphorus pentoxide to give β-CDOTs (4.5 g). T.l.c. (solvent A) showed: R_C (relative to β-CD), β-CDOTs, 1.6. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), β-CDOTs, 0.56.

FAB MS $M+H^+$ requires 1289, found 1289; $M+Na^+$ requires 1311, found 1311. ^1H n.m.r. (D_6DMSO) δ_H 2.47, Me; 3.2 - 4.0, 4.2 - 5.1, 69H; 7.6, 7.9, ABq, J_{AB} = 2.2 Hz, ArH. ^{13}C n.m.r. (D_6DMSO, D_2O) δ_C 25.1_0, 63.9_8, 75.8_4, 76.3_9, 76.7_9, 81.9_7, 82.4_1, 82.8_1, 85.6_0, 105.9_8, 133.5_2.

EXAMPLE 15

6^A-Azido-6^A-deoxy-β-cyclodextrin (β-CDN_3)

β-CDOTs (2 g) and sodium azide (2 g) were dissolved in water (60 ml) and the solution heated on a boiling water bath for 90 minutes. The solution was concentrated *in vacuo* to approximately 10 ml. TCE (1 ml) was added and the mixture shaken to give a white precipitate. After standing for 60 minutes, the solid was collected by vacuum filtration, washed with small amounts of ice cold water (ca. 3 x 2 ml) and dried. The collected solid was heated in water (60 ml) until all of the product dissolved. The TCE was removed and the solution evaporated to dryness to give the crude product. Recrystallization from boiling water gives β-CDN_3 as clear colorless crystals (1.4 g). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CDN_3, 1.4. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), β-CDN_3, 0.66.

Found: N 3.57. $C_{42}H_{69}N_3O_{34}$ requires: N 3.53%. FAB MS $M+H^+$ requires 1160, found 1160; $M+Na^+$ requires 1182, found 1182. υ_{max} 2107 cm^{-1}. ^1H n.m.r. (D_6DMSO) δ_H 3.2 - 5.9, 69H. ^{13}C n.m.r. (D_6DMSO) δ_C 55.0, 63.9, 74.2, 76.2, 77.0, 85.5, 87.0, 105.9, 106.8.

Examples 16 to 18 illustrate alternative syntheses for preparing 6^A-amino-6^A-deoxy-β-cyclodextrin.

EXAMPLE 16

6^A-Amino-6^A-deoxy-β-cyclodextrin (β-$CDNH_2$)

β-CDN_3 (770 mg) was dissolved in water (150 ml) and palladium black (250 mg) was added. The mixture was shaken (18 hours) at 30 psi on a Parr hydrogenator. After venting the hydrogen, a small volume of TCE (0.1 ml) was added to the mixture

which was then shaken for 30 seconds. After standing for 15 minutes the solution was filtered (Whatman № 1 filter paper) and the clear colorless solution was evaporated to dryness to give β-$CDNH_2$ (660 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-$CDNH_2$, 0.6. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), β-$CDNH_2$, 1.27.

Found: N 1.34. $C_{42}H_{71}NO_{34}$ requires: N 1.24%. FAB MS $M+H^+$ requires 1134, found 1134. ^1H n.m.r. (D_6DMSO) δ_H 3.0 - 6.1, 69H; 8.0 - 8.3, NH_2. ^{13}C n.m.r. (D_2O; dioxane std) δ_C 42.12, t, 61.26, t, 72.53, d, 72.85, d, 73.03, d, 74.06, d, 81.91, d, 82.10, d, 83.13, d, 102.62, 102.80, d.

EXAMPLE 17

β-CDOTs (340 mg) was dissolved in 0.880 ammonia solution (10 ml) and left standing at room temperature for 2 weeks. After removing the ammonia *in vacuo* the residue was poured into acetone (50 ml). The precipitate was collected by gravity filtration and dried *in vacuo* to give β-$CDNH_2$ (301 mg). T.l.c. and HPLC showed a spot corresponding to β-$CDNH_2$.

EXAMPLE 18

Anhydrous β-CDOTs (1.0 g) was dissolved in dry DMF (50 ml) in a 400 ml pressure vessel. Condensed ammonia (100 ml) was added carefully and the vessel sealed. The pressure increased to 150 psi as the reaction mixture warmed from -33°C to 22°C (room temperature). The reaction mixture was stirred at room temperature for 3 days. The ammonia was vented off and the DMF solution was dried *in vacuo*. The residue was dissolved in water (10 ml) and evaporated *in vacuo* to dryness twice to remove any residual DMF. The residue (0.895 g) was dissolved in 20% aqueous ammonia (10 ml) and dropped into acetone (200 ml). The precipitate was collected by vacuum filtration and rinsed with acetone (5 ml) and ether (5 ml) to give 450 mg of white powder. T.l.c. and HPLC showed one spot and one peak corresponding to β-$CDNH_2$.

EXAMPLE 19

6^A-Amino-6^A-deoxy-β-cyclodextrin hydrochloride (β-CDNH$_3$Cl)

Concentrated hydrochloric acid was added dropwise to a stirred suspension of β-CDNH$_2$ (1 g) in water (5 ml) at 0°C until the solution reached ~pH 2 (ca. 5 drops). The cold solution was filtered through a plug of cotton wool into acetone (150 ml) and the precipitate collected by vacuum filtration and air dried to give 820 mg of a white powder. The crude salt was dissolved in water (10 ml) and left to stand in a sealed vessel also containing acetone (25 ml). After 3 weeks at room temperature a white precipitate formed. This was collected by vacuum filtration, rinsed with acetone (20 ml) and ether (20 ml) and air dried to give β-CDNH$_3$Cl (555 mg).

EXAMPLE 20

2^A-O-*p*-Toluenesulfonyl-β-cyclodextrin (β-2CDOTs)

Step 1: 3-Nitrophenyl *p*-toluenesulfonate (TsNP) can be prepared as follows. A solution of *p*-toluenesulfonyl chloride (1.91 g) and *m*-nitrophenol (1.39 g) in pyridine (10 ml) was stirred overnight. Ethyl acetate (50 ml) was added and the mixture washed with brine (2 x 50 ml), dried over magnesium sulfate and concentrated *in vacuo*. The residue was recrystallized from ethyl acetate - petroleum ether to give brown crystals of TsNP (1.81 g), m.p. 112 - 114°C. ^{1}H n.m.r. δ_H 2.5 (s, 3H, C<u>H</u>$_3$); 7.3 - 7.9 (m, 8H, Ar-<u>H</u>).

Step 2: Anhydrous β-CD (7.02 g) was added to a solution of TsNP (1.81 g) in DMF (60 ml). Aqueous pH 11.5 carbonate buffer (0.2 M, 0.42 ml) was added and the mixture stirred at 60°C for 1 hour. The reaction mixture was neutralized with 1 N hydrochloric acid and unreacted β-CD was precipitated by the addition of acetone (500 ml). The mixture was filtered, the filtrate concentrated to a small volume (ca. 3 ml) and acetone (100 ml) was added. The precipitate was collected by filtration, washed with acetone and dried to give the crude product (1.19 g). HPLC of the crude product using a 70% CH$_3$CN-H$_2$O eluant showed: t_R (relative to β-CD) salt, 0.13; β-3CDOTs, 0.21; β-2CDOTs, 0.28;

βCD, 1.0 (Assignments of β-3CDOTs and β-2CDOTs may be interchangeable). Each component was separated by preparative HPLC using a C_{18} μ-bondapack column to give respective yields of 603, 27 and 175 mg. This experiment has been repeated a number of times where no material with t_R 0.21 has been present.

β-2CDOTs: FAB MS $M+H^+$ requires 1289 found 1289; $M+Na^+$ requires 1311 found 1311. ^1H n.m.r. (D_6DMSO) δ_H 2.51, Me; 3.2 - 5.1, 5.6 - 6.1, 69H; 7.5, 7.9, ABq, J_{AB} = 8.3 Hz, ArH. ^{13}C n.m.r. (D_6DMSO) δ_C 25.1_3, 63.6_6, 73.2_6, 75.5_8, 76.0_1, 76.3_3, 76.6_3, 76.9_9, 83.6_2, 84.9_0, 85.5_0, 102.0_9, 105.6_7, 132.0_1, 133.7_7, 148.8_3. β-3CDOTs: ^1H n.m.r. (D_6DMSO) δ_H 2.38, Me; 3.3 - 5.0, 5.6 - 6.0, 69H; 7.1, 7.5, ABq, J_{AB} = 8.1 Hz, ArH.

EXAMPLE 21

2^A-Azido-6^A-deoxy-β-cyclodextrin (β-2CDN$_3$)

A solution of β-2CDOTs (62 mg) and sodium azide (62 mg) in water (8 ml) was heated on a boiling water bath for 2 hours. The water was removed *in vacuo* and the resulting residue separated by preparative HPLC to give β-2CDN$_3$ (10 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-2CDN$_3$, 1.37. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), β-2CDN$_3$, 0.66. υ_{max} 2116 cm^{-1}. ^{13}C n.m.r. (D_2O) δ_C 60.6_7, 61.5_6, 62.6_9, 70.9_7, 72.7_1, 72.9_3, 73.1_2, 73.5_6, 74.0_1, 74.3_9, 76.2_2, 78.4_8, 81.0_7, 81.7_8, 82.0_2, 82.2_3, 101.9_5, 102.3_6, 102.5_7, 102.9_5, 104.2_3.

EXAMPLE 22

2^A-O-(3-Nitrobenzenesulfonyl)-α-cyclodextrin (α-2CDONs)

3-Nitrobenzenesulfonyl chloride (30 g) was added in one portion to a vigorously stirred solution of α-CD (30 g) in water (105 ml), adjusted to pH 12 by addition of 2 M sodium hydroxide. When the solution became neutral (approximately 5 minutes) the mixture was gravity filtered (Whatman № 1 filter paper) and the filtrate loaded onto a Sephadex® G-15 column. Elution with water gave the desalted α-2CDONs (33 g). T.l.c. (solvent B) showed: R_C (relative to α-CD), α-2CDONs, 2.0. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-2CDONs, 0.39. FAB MS

$M+Na^+$ requires 1180 found 1180. The partially purified product (1.0 g) was dissolved in 15% aqueous methanol (20 ml), filtered (0.22 μm) and loaded, via the pump, onto a 19 x 150 mm C_{18} μ-Bondapack HPLC column. This was eluted with 15% aqueous methanol at 15 $ml.min^{-1}$ and gave pure α-2CDONs (25 - 35 minutes, 420 mg, 42%). ^1H n.m.r. (D_6DMSO, 2.6) δ_H 3.3 - 3.9; 4.1 - 4.6, 6 x OH; 4.8 - 5.0; 6 x Cl-H; 5.4 - 5.8, 11 x OH; 8.0 - 8.8, ArH. ^{13}C n.m.r. (D_2O) δ_C 61.2_7, 61.5_4, 68.0_0, 71.1_6, 72.8_4, 73.0_9, 73.2_9, 73.8_2, 74.3_8, 74.5_8, 81.2_6, 82.4_2, 82.8_7, 99.8_2, 102.5 , 102.63, 124.4_6, 130.5_7, 132.8_2, 135.8_1, 137.7_7, 149.1_4.

EXAMPLE 23

Cyclodextrin epoxide from 2-o-(4-Nitrobenzenesulfonyl)-α-cyclodextrin

Pure α-2CDONs was stirred with a 10% aqueous ammonium bicarbonate solution at 60°C overnight. The solvent was removed by distillation under reduced pressure to give a solid. HPLC of the product using a 70% acetonitrile - water eluant showed: t_R (relative to α-CD), 0.39. FAB MS 954.

EXAMPLE 24

(2^S, 3^S)-3^-Amino-3^-deoxy-α-cyclodextrin (α-3CDNH$_2$)

Desalted α-2CDONs (33 g) was dissolved in 33% ammonia solution (150 ml) and the solution was stirred at 60° overnight. The solution was concentrated *in vacuo* to 100 ml and cyclohexane (6 ml) added and the mixture stirred to precipitate α-cyclodextrin. The precipitate was collected by centrifugation and washed twice with cold water. The precipitate was suspended in water and heated to remove the cyclohexane. Removal of the solvent *in vacuo* gave α-3CDNH$_2$ (18 g). T.l.c. (solvent B) showed: R_C (relative to α-CD), α-3CDNH$_2$, 0.35. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-3CDNH$_2$, 1.27. FAB MS $M+H^+$ requires 972, found 972.

EXAMPLE 25

Crude α-cyclodextrin epoxide was dissolved in 28% ammonia solution (10 ml). This solution was stirred at 60° overnight and

the solvent removed *in vacuo* to give crude 3-amino-3-deoxy-α-cyclodextrin. This was purified by Sephadex G-15 chromatography using a water eluant. T.l.c. (solvent B) of the product showed: R_C (relative to α-CD), 0,35. HPLC of the product using a 70% acetonitrile - water eluant showed: t_R (relative to α-CD), 1.27.

FABMS $M+H^+$ requires 972, found 972. ^{13}C n.m.r. (D_2O, dioxane std) δ_C 53.1, 61.3, 61.6, 61.8, 62.0, 70.5, 72.4, 72.8, 73.0, 73.2, 73.5, 74.0, 74.3, 74.5, 77.1, 79.7, 81.1, 82.0, 82.5, 82.6, 101.0, 101.6, 102.0, 102.5, 102.9, 103.1, 105.2.

EXAMPLE 26

3-Nitrobenzenesulfonyl chloride (1.4 g) was added in one portion to a vigorously stirred solution of α-cyclodextrin (1.4 g) in water (5 ml), adjusted to pH 12 by addition of 2M sodium hydroxide. After 5 minutes the mixture had become neutral and unreacted 3-nitrobenzenesulfonyl chloride was filtered off. The filtrate was diluted with water (5 ml) and ammonium bicarbonate (1 g) was added. The mixture was stirred at 60° for 4 hours. The water was evaporated *in vacuo* and the residue dissolved in 28% ammonia solution (10 ml). This solution was stirred at 60° overnight and the solvent removed *in vacuo* to give crude α-3CDNH$_2$. T.l.c. (solvent B) showed: R_C (relative to α-CD), α-3CDNH$_2$, 0.35. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-3CDNH$_2$, 1.27. FAB MS $M+H^+$ requires 972, found 972.

EXAMPLE 27

6^A-Deoxy-6^A-iodo-α-cyclodextrin (α-CDI)

A solution of α-CDOTs (1 g) and sodium iodide (1 g) in water (100 ml) was heated on a boiling water bath for 2 hours. The solution was evaporated *in vacuo*. The solid was dissolved in a minimum volume of boiling water and allowed to recrystallize. Pale yellow crystals (490 mg) of α-CDI were collected. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-CDI, 0.58. FAB MS $M+H^+$ requires 1083, found 972; $M+Na^+$ requires 1105, found 1105.

EXAMPLE 28

6^A-Deoxy-6^A-iodo-β-cyclodextrin (β-CDI)

β-CDOTs (100 mg) and sodium iodide (100 mg) were heated together in DMF (1 ml) at 80°C overnight. The solution was poured into acetone (10 ml) and the solid centrifuged down. After recrystallization from water (1 ml) the recovered solid shows only a single spot by t.l.c.: R_C (relative to β-CD), β-CDI, 1.57. FAB MS $M+H^+$ requires 1245, found 1245; $M+Na^+$ requires 1267, found 1267.

EXAMPLE 29

2^A-O-Acetyl-α-cyclodextrin (α-2CDOAc)

Step 1: 3-Nitrophenyl acetate (AcNP) can be prepared as follows. Magnesium turnings (1.2 g) were added to a solution of 3-nitrophenol (6.95 g), benzene (40 ml) and acetyl chloride (4 g) and the solution heated for 1 hour at 90°C. The reaction mixture was diluted with ether and decanted from unreacted magnesium. The ether layer was washed successively with water, dilute NaOH and water. The organic layer was dried (Na_2SO_4) and the solvent removed *in vacuo* to give AcNP. m.p. 55-56°C. ^1H n.m.r. δ_H 2.4, s, 3H, $\underline{H}_3C$-CO-; 7.4 - 8.4, m, 4H, Ar$\underline{H}$. $\upsilon_{c=o}$ 1770 cm^{-1}.

Step 2: To a solution of α-CD (0.97 g) dissolved in a pH 9.8 carbonate buffer (62.5 ml) was added 0.2 M AcNP in acetonitrile (4.65 ml). After stirring for 13 minutes, dilute HCl was added until the reaction mixture reached pH 4.9. The reaction mixture was cooled to room temperature and any phenol formed extracted with ether. The aqueous layer is concentrated *in vacuo* to yield a white powder. ^1H n.m.r. analysis of this powder showed α-2CDOAc to be present in an estimated 5% yield. $\upsilon_{c=o}$ 1734 cm^{-1}. FAB MS $M+Na^+$ requires 1038, found 1038.

EXAMPLE 30

6^A-O-Acetyl-α-cyclodextrin (α-CDOAc)

A mixture of α-CDOTs (1.13 g) and cesium acetate (0.19 g) in dry DMF (5 ml) was heated at 100°C for 24 hours. The reaction

mixture was cooled and acetone was added until precipitation was complete. The precipitate was collected by vacuum filtration and washed with acetone. HPLC using a 70% CH_3CN - H_2O eluant showed: t_R (relative to α-CD), 0.22, 0.36, 0.62, 1.0. The component at t_R 0.62 was isolated by column chromatography using Sephadex® G-15 and 5:1 CH_3CN - water eluant and was identified as α-CDOAc.

FAB MS $M+H^+$ requires 1015 found 1015; $M+Na^+$ requires 1037 found 1037. $\upsilon_{c=o}$ 1735 cm^{-1}. ^1H n.m.r. (D_6DMSO) δ_H 2.1, CH_3, 3.1 - 5.9, 53H. ^{13}C n.m.r. (D_6DMSO) δ_C 24.5_5; 63.9_5; 67.0_0; 72.8_4, 76.0_6, 77.1_9, 86.0_1, 86.2_7, 105.5_5, 105.9_1.

EXAMPLE 31

6^A-O-Acetyl-β-cyclodextrin (β-CDOAc)

β-CDOTs (1.3 g) and cesium acetate (0.6 g) were dissolved in DMF (10 ml) and stirred at 110 - 120°C for 20 hours. After cooling the reaction mixture, excess acetone was added until precipitation was complete and the product was collected by vacuum filtration (yield 1.0 g). Recrystallization from water followed by Sephadex® G15 chromatography (5:1 acetonitrile : water) of the crude product gave β-CDOAc (0.4 g). HPLC using a 70%CH_3CN- H_2O eluant showed: t_R (relative to β-CD), β-CDOAc, 0.6.

$\upsilon_{c=o}$ 1736 cm^{-1}. FAB MS $M+H^+$ requires 1177 found 1177. ^1H n.m.r. (D_6DMSO) δ_H 2.1, Me, 3.1 - 6.0, 69H. ^{13}C n.m.r. (D_6DMSO) δ_C 24.4_9, 63.5_1, 63.9_4, 67.3_2, C6A; 72.8_8, 76.0_4, 76.4_1, 77.0_3, 85.1_0, 85.6_1, 85.6_6, 86.3_3, 105.9_3, 106.4_5, 174.7_3.

EXAMPLE 32

6^A-N-Acetyl-6^A-amino-6^A-deoxy-α-cyclodextrin (α-CDNAc)

To a solution of α-$CDNH_2$ (100 mg) dissolved in dry methanol (7.5 ml), was added acetic anhydride (1.5 ml) and the reaction mixture stirred for 6 hours. Water (1.5 ml) was added followed by acetone (20 ml) to precipitate the product. The precipitate was separated by filtration through fluted № 50 qualitative Whatman filter paper and washed with acetone to give crude product (146 mg). T.l.c. (solvent A) of the crude product

showed: R_C (relative to α-CD), α-CDNH$_2$, 0.7; α-CDNAc, 1.1. HPLC of the crude product using a 75% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), unknown, 0.6; α-CDNAc, 0.9; α-CDNH$_2$, 1.2.

α-CDNAc was separated by preparative HPLC. FAB MS $M+H^+$ requires 1014, found 1014; $M+Na^+$ requires 1036, found 1036.

Examples 33 to 42 illustrate preparation of prodrugs in which Ibuprofen (α-methyl-4-(2-methylpropyl)-benzeneacetic acid) or Naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid) are covalently bonded directly to a cyclodextrin through ester linkages.

EXAMPLE 33

2^A-O-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-α-cyclodextrin (α-2CDOIb)

Step 1: 3-Nitrophenyl α-methyl-4-(2-methylpropyl)-benzeneacetate (IbNP) can be prepared as follows. A mixture of α-methyl-4-(2-methylpropyl)-benzeneacetic acid (1.03 g) and 3-nitrophenol (0.70 g) were dissolved in dry ethyl acetate (100 ml). The solution was cooled to 0°C, N,N'-dicyclohexylcarbodiimide (1.68 g,) was added and the reaction for one hour. The reaction mixture was allowed to warm to room temperature and stirred overnight. Dicyclohexylurea was removed by filtration and the filtrate evaporated *in vacuo* to yield the crude ester as a glassy thick liquid (1.27 g). This was purified by flash chromatography (Matrex Silica Gel 50 μm) using hexane - ethyl acetate gradient elutions to give IbNP as an oil (1.08 g). $\upsilon_{c=o}$ 1760 cm^{-1}. Found: C 69.77, H 6.60, N 4.32. $C_{19}H_{21}NO_4$ requires: C 69.71, H 6.47, N, 4.28%. EI MS found 327.14585, requires 327.14706. ^1H n.m.r. ($CDCl_3$; TMS standard) δ_H 0.91, d, (J 7 Hz), 6H, $(C\underline{H}_3)_2CH$-; 1.62, d, (J 7 Hz), 3H, -$C\underline{H}_3$; 1.87, m, (J 7 Hz), 1H, $(CH_3)_2C\underline{H}$-; 2.47, d, (J 7Hz), 2H, -$C\underline{H}_2$-; 3.97, q, (J 7Hz), 1H, H_3C-$C\underline{H}$, 7.0 - 8.1, m, 8H, Ar$\underline{H}$. ^{13}C n.m.r. ($CDCl_3$) δ_C 18.3, 22.3, 30.7, 45.1, 117.1, 120.5, 127.1, 127.8, 129.6, 129.8, 136.5, 141.1, 148.6, 151.1, 172.4.

Step 2: α-CD (0.97 g) was dissolved in a 1:1 mixture of aqueous NaOH (4×10^{-4} M) - acetonitrile (80 ml). IbNP (0.33 g) in acetonitrile (10 ml) was added gradually with stirring, and NaOH (0.1 M) was added until the reaction mixture reached pH 10. After being left to stir for 100 minutes, hydrochloric acid (4 N) was added to the reaction mixture until it reached pH 2. Chloroform (50 ml) was added to the reaction mixture and the organic layer was separated, dried (Na_2SO_4) and the solvent removed *in vacuo*. Ether was used to dissolve the soluble 3-nitrophenol, leaving behind the crude product (0.2 g). HPLC of the crude product using a 70% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), 0.1, 0.15, 0.2, 0.25, 1.0.

The component at t_R 0.25 was purified by preparative HPLC and identified as α-2CDOIb. FAB MS $M+H^+$ requires 1161 found 1161; $M+Na^+$ requires 1183, found 1183. $\upsilon_{c=o}$ 1728 cm^{-1}.

EXAMPLE 34

2^A-O-(α-methyl-4-(2-methylpropyl)-benzeneacetyl))-β-cyclodextrin (β-2CDOIb)

β-CD (1.14 g) was dissolved in a 1:1 mixture of aqueous NaOH (4×10^{-4} M) - acetonitrile (100 ml). IbNP (Example 33, Step 1; 0.323 g) in acetonitrile (10 ml) was gradually added with stirring and NaOH (0.1 M) was added until the reaction mixture reached pH 10. After being left to stir at room temperature for 100 minutes, hydrochloric acid (4 M) was added to the reaction mixture until it reached pH 2.5. Water was removed by distillation at 40°C under reduced pressure. The solid was redissolved in water and filtered, and the filtrate extracted with ether to remove m-nitrophenol. The water was removed by distillation under reduced pressure to give a solid. Preparative HPLC of this solid using a 70% CH_3CN-H_2O enabled the purification of β-2CDOIb: t_R (relative to β-CD), β-2CDOIb, 0.65.

FAB MS $M+H^+$ requires 1323, found 1323. $\upsilon_{c=o}$ 1728 cm^{-1}

EXAMPLE 35

2^A-O-((S)-6-methoxy-α-methyl-2-naphthaleneacetyl)-β-cyclodextrin (β-2CDONp)

Step 1: 3-Nitrophenyl (S)-6-methoxy-α-methyl-2-naphthaleneacetate (NpNP) can be prepared as follows. A mixture of (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid (1.0 g) and 3-nitrophenol (0.6 g) were dissolved in dry ethyl acetate (100 ml). The solution was cooled to 0°C, DCC (1.0 g) was added and the reaction for one hour. The reaction mixture was allowed to warm to room temperature and stirred overnight. DCU was removed by filtration and the filtrate evaporated *in vacuo* to yield the crude ester as a solid. The solid was dissolved in chloroform (5 ml) and ether (40 ml) and filtered to remove any remaining DCU. The filtrate was evaporated *in vacuo* and the residue recrystallized from ether (6 ml) and hexane (40 ml) to give NpNP as a solid (0.88 g). Mp 79°C. $\upsilon_{c=o}$ 1770 cm^{-1}. Found: C 68.11, H 4.82, N 4.03. $C_{20}H_{17}NO_5$ requires: C 68.37, H 4.88, N 3.99%. EI MS found 351.11024, requires 351.11067. ^1H n.m.r. ($CDCl_3$; TMS standard) δ_H 1.71, d, (J 7 Hz), 3H, $C\underline{H}_3CH$-; 3.9, s, 3H, $C\underline{H}_3O$-; 4.13, q, (J 7 Hz), $CH_3C\underline{H}$-; 7.1-8.1, m, 10H, ArH. ^{13}C n.m.r. ($CDCl_3$) δ_C 18.4, 45.6, 55.4, 105.7, 117.3, 119.3, 120.7, 125.9, 126.2, 127.6, 127.9, 129.0, 129.3, 129.9, 134.0, 134.5, 151.1, 157.9, 172.6.

Step 2: To a solution of β-CD (1.13 g) in aqueous DMF (10 ml, 1:3) was added NpNP (0.351 g) and the reaction mixture was stirred at 100 - 110°C for 24 hours. After this time analysis of a portion of this mixture by HPLC showed: t_R (relative to β-CD), β-2CDONp, 0.36; β-CD, 1.0. The reaction was heated for a further 24 hours, the reaction mixture cooled and acetone was added until precipitation was complete. The precipitate was collected by gravity filtration (Whatman № 1 qualitative filter paper) and recrystallized from water to give a solid which was poorly soluble in water. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), 0.36, 1.00.

EXAMPLE 36

6^A-O-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-α-cyclodextrin (α-CDOIb)

α-CDOTs (1 g) and potassium α-methyl-4-(2-methylpropyl)-benzeneacetate (600 mg) were heated in DMF (5 ml) at 100°C for 24 hours. The reaction mixture was cooled and acetone was added until precipitation was complete. The mixture was filtered and the solid was washed with acetone and collected. The product mixture was chromatographed on Sephadex® G15 and the major product (α-CDOIb) was isolated together with a small amount of α-CD (yield 0.7 g). HPLC using a 70% CH_3CH-H_2O eluant showed: t_R (relative to α-CD), α-CDOIb, 0.25.

υ_{max} 1728 cm^{-1}. FAB MS M+Na^+ requires 1183 found 1183; M+K^+ requires 1200 found 1200. ^1H n.m.r. (D_6DMSO) δ_H 0.9 - 1.0, d, Me_2; 1.4 - 1.5, d, (+)-Me, (-)-Me; 1.85 - 1.95, m, $CHMe_2$; 2.5 - 2.6, d, CH_2; 3.2 - 5.8, 59H; 7.1 -7.4, ArH. ^{13}C n.m.r. (D_6DMSO) δ_C 22.5_2, 26.1_6, 33.5_4, 48.0_1, 48.2_2, 63.8_8, 67.0_0, 68.0_0, 72.9_4, 76.0_5, 77.1_6, 85.9_6, 105.9_9, 131.1_0, 132.9_2, 141.7_2, 143.7_2, 171.7_2, 183.5_7.

The reaction was repeated using sodium and cesium salts of α-methyl-4-(2-methylpropyl)-benzeneacetate to give the same product mixture.

EXAMPLE 37

6^A-O-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-β-cyclodextrin (β-CDOIb)

β-CDOTs (200 mg) and the sodium α-methyl-4-(2-methylpropyl)-benzeneacetate (60 mg) were stirred and heated in DMF (2 ml) at 100°C for 20 hours. The reaction mixture was cooled and acetone added until precipitation was complete. The mixture was filtered and the solid was washed with acetone and recrystallized from water to give crude product. Two components were isolated together by column chromatography using Sephadex® G-15 and 5:1 acetonitrile - water as the eluant. HPLC of the crude product using a 70% acetonitrile - water eluant showed: t_R (relative to

β-CD), 0.32 (β-CDOIb⁻), 0.38 (β-CDOIb⁺).

$\upsilon_{c=o}$ 1735 cm^{-1}. FAB MS $M+H^+$ requires 1323 found 1323; $M+Na^+$ requires 1345 found 1345. ^1H n.m.r. (D_6DMSO) δ_H 0.9 - 1.0, 2 x d, 2 x Me_2; 1.4 - 1.5, d, (+)-Me, (-)-Me; 1.85 - 2.0, m, $CHMe_2$; 2.5 - 2.6, d, CH_2; 3.0 - 6.0, 59H; 7.1 -7.3, ArH. ^{13}C n.m.r. (D_6DMSO) δ_C 21.6_3, 22.0_9, 26.1_0, 26.2_3, 33.6_0, 48.1_0, 48.2_3, 63.9_0, 76.0_8, 76.4_4, 77.0_8, 85.5_5, 105.9_4, 131.0_8, 132.9_3. The two isomers were separated by preparative HPLC. FAB MS indicated that the two products were of the same mass, $M+H^+$ 1323, $M+Na^+$ 1345. ^1H n.m.r. allowed identification of each product as isomers of β-CDOIb.

The reaction was repeated using potassium and cesium salts of α-methyl-4-(2-methylpropyl)-benzeneacetate to give the same isomeric mixture.

EXAMPLE 38

Methyl α-methyl-4-(2-methylpropyl)-benzeneacetate (IbOMe)

To a solution of α-methyl-4-(2-methylpropyl)-benzeneacetic acid (1.05 g, 5.1 mmol) in methanol (200 ml) was added thionyl chloride (0.8 ml). The mixture was left to stir at room temperature for 4.5 hours and the solvent removed *in vacuo*. The residue was dissolved in dichloromethane (20 ml) and extracted with 0.25 M sodium bicarbonate (20 ml). The organic layer was dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the methyl ester as a clear colorless oil (0.89 g).

υ_{max} 1741, 1513 cm^{-1}. ^1H n.m.r. ($CDCl_3$, TMS standard) δ_H 0.896, d, $J_{1,2}$ 7 Hz, $(H1)_6$; 1.484, d, $J_{8,9}$ 7 Hz, $(H9)_3$; 1.843, heptet, $J_{1,2}$ 7 Hz, $J_{2,3}$ 7 Hz, H2; 2.4429, d, $J_{2,3}$ 7 Hz, H3; 3.652, s, $(H11)_3$; 3.696, q, $J_{8,9}$ 7 Hz, H8; 7.093, 7.193, ABq of t, J_{AB} 8 Hz, $J_{AX} = J_{BX} = 2$ Hz, $(H5)_2$, $(H6)_2$.

On addition of the chiral shift reagent, the tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] derivative of Europium ($Eu(hfc)_3$), to the $CDCl_3$ solution, resonances shifted,

H8 and H9 split into 2 quartets and 2 doublets respectively.

EXAMPLE 39

(R)-α-Methyl-4-(2-methylpropyl)-benzeneacetic acid ((R)-Ibuprofen)

Horse liver acetone powder (0.89 g) was added to a suspension of IbOMe (0.89 g, 4.04 mmol) in 0.2 M phosphate buffer (36 ml). The reaction was followed by t.l.c. (5% acetic acid, 10% ethyl acetate, 85% hexane). Conversion of the 50% of the IbOMe (R_f 0.37) to Ibuprofen (R_f 0.15) appeared complete after 11 hours. The reaction was quenched by adding 1 M hydrochloric acid until the solution reached pH 2. Ether (20 ml) was added to the reaction mixture and the two layers centrifuged (3000 r.p.m., 15 minutes). The solid collected at the interface of the organic and aqueous layers and was separated by carefully decanting both liquid layers. The organic layer was separated and the aqueous layer re-extracted with ether. The emulsion formed was discarded and the aqueous layer kept. The combined ether layers were extracted with saturated sodium bicarbonate (2 x 15 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to give and oil (IbOMe, 540 mg). The aqueous layer was adjusted to pH 1 with concentrated hydrochloric acid and extracted with dichloromethane (2 x 20 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the resolved (R)-Ibuprofen (81 mg). The enantiomeric purity of the (R)-Ibuprofen was confirmed by ^1H n.m.r. of its corresponding methyl ester (Example 40) in the presence of the chiral shift reagent Eu(hfc)$_3$. H8 and H9 resonances shifted significantly and did NOT split.

EXAMPLE 40

6^A-O-((R)-α-methyl-4-(2-methylpropyl)-benzeneacetyl)-β-cyclodextrin (β-CDOIbR)

Step 1: To a solution of (R)-α-methyl-4-(2-methylpropyl)-benzeneacetic acid (Example 39, 80 mg, 0.4 mmol) in dry methanol (2 ml) was added cesium carbonate (70 mg, 0.22 mmol) in portions over a 5 minute period with stirring. The mixture was allowed to stir at room temperature for a further 55 minutes and then

dried *in vacuo*. The resulting green oily residue was twice dissolved in dry ether (1 ml) and dried *in vacuo*. The oil was dried *in vacuo* over P_2O_5 to give cesium (R)-α-methyl-4-(2-methylpropyl)-benzeneacetate as a green oil (127 mg).

Step 2: To a mixture of cesium (R)-α-methyl-4-(2-methylpropyl)benzeneacetate (0.127 g) in dry DMF (2 ml) was added β-CDOTs (0.45 g,). The mixture was stirred at 100°C for 20 hours and allowed to cool. Acetone (3 ml) was added to the solution until precipitation appeared complete. The resulting brown solid was collected by vacuum filtration and suspended in hot water (2 ml). The white solid was collected by vacuum filtration and dried *in vacuo* over P_2O_5 to give β-CDOIb- in yield 188 mg. HPLC using a 70% CH_3-H_2O eluant showed: t_R (relative to β-CD), β-CDOIbR, 0.32.

EXAMPLE 41

6^A-O-((S)-6-methoxy-α-methyl-2-naphthaleneacetyl)-α-cyclodextrin (α-CDONp)

To a solution of α-CDOTs (1.13 g) in dry DMF was added cesium (S)-6-methoxy-α-methyl-2-naphthaleneacetate (0.36 g). The mixture was stirred at 100°C for 24 hours, cooled and acetone (150 ml) was added until precipitation was complete. The precipitate was collected by gravity filtration (Whatman № 1 qualitative filter paper) and washed with acetone to give a solid which was poorly soluble in water. HPLC of the crude product using a 70% acetonitrile - water eluant showed: t_R (relative to α-CD), unknown, 0.2; α-CDONp, 0.27; α-CD, 1. The component at t_R 0.27 was isolated by column chromatography using Sephadex® G-15 and 5:1 acetonitrile - water eluant and was identified as α-CDONp.

$\upsilon_{c=o}$ 1728 cm^{-1}. FAB MS $M+Na^+$ requires 1207 found 1207. ^1H n.m.r. (D_6DMSO) δ_H 1.45 - 1.55, d, Me; 3.1 - 5.8, 62H; 7.2 - 8.1, ArH. ^{13}C n.m.r. (D_6DMSO) δ_C 22.8_0, q; $48._2$, d; 59.1_0, q, ; 63.8_9, t; 67.9_0, t; 72.7_7,d, 76.0_5, d, 77.1_5, d, 85.7_4, d, 85.9_7, d, 86.1_2, d, 105.6_1, d, 106.0_0, d, 109.6_7, d, 122.5_9, d, 129.3_6, d,

130.3$_6$, d, 132.3$_3$, d, 133.1$_9$, d, 137.1$_6$, d, 139.5$_8$, s, 139.7$_1$, s, 161.1$_2$, s, 171.3$_4$, s, 177.8$_1$, s.

EXAMPLE 42

6^A-O-((S)-6-Methoxy-α-methyl-2-naphthaleneacetyl)-β-cyclodextrin (β-CDONp)

To a solution of β-CDOTs (1.29 g) in dry DMF (5 ml) was added the cesium (S)-6-methoxy-α-methyl-2-naphthaleneacetate (0.36 g). The mixture was stirred for 18 hours at 100 - 110°C, cooled and acetone added until precipitation was complete. The precipitate (2 g) was collected by gravity filtration (Whatman № 1 qualitative filter paper) and recrystallized from water. HPLC using 70% CH_3CN-H_2O as eluant showed: t_R (Relative to β-CD), β-CDONp, 0.28; β-CD, 1. The component at t_R 0.28 was isolated by column chromatography and 5:1 acetonitrile - water eluant and was identified as β-CDONp.

$\upsilon_{c=o}$ 1736 cm^{-1}. FAB MS M+H$^+$ requires 1347, found 1347. ^{1}H n.m.r. (D_6DMSO) δ_H 1.45 - 1.55, d, Me; 3.0 - 6.0, 72H; 7.2 - 8.0, ArH. ^{13}C nmr (D_6DMSO) δ_C 22.7$_3$, Me; 48.2$_3$, CH; 59.1$_1$, OMe; 63.5$_6$, 63.9$_0$, C6^{B-F}; 67.6$_0$, C6^A; 72.7$_9$, 72.7$_9$, 76.0$_2$, 76.3$_8$, 77.0$_0$, 85.0$_5$, 85.5$_5$, 85.6$_1$, 85.9$_4$, 105.9$_2$, 106.2$_5$, 109.6$_7$, 122.5$_6$, 129.4$_9$, 130.4$_4$, 130.6$_2$, 132.3$_3$, 133.2$_3$, 137.1$_6$, 139.7$_1$, 161.1$_1$, 177.6$_1$.

Examples 43- 50 illustrate preparation of prodrugs in which Ibuprofen (α-methyl-4-(2-methylpropyl)-benzeneacetic acid) or Naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid) is covalently bonded directly to a cyclodextrin through amide linkages.

EXAMPLE 43

6^A-Deoxy-6^A-N-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-α-cyclodextrin (α-CDNIb)

Step 1: α-Methyl-4-(2-methylpropyl)-benzeneacetic acid anhydride (Ib_2O) was prepared as follows. α-Methyl-4-(2-methylpropyl)-benzeneacetic acid (1500 mg) was dissolved in dry ether (60 ml) and DCC (750 mg) was added. Insoluble DCU forms

immediately. After 1 hour the ether was removed *in vacuo* and ethyl acetate (600 ml) was added to the residue. The product only slowly dissolved in ethyl acetate so sufficient agitation and time was required to ensure solubilization. The insoluble DCU was filtered off and ethyl acetate removed *in vacuo* to give Ib_2O (1.35 g). υ_{max} 1703, 1747 cm^{-1}. ^1H n.m.r. ($CDCl_3$) δ_H 1.1, d, 12H; 1.5, d, 6H; 2.5, d, 4H; 3.8, q, 2H; 7.2, 8H.

Step 2: α-$CDNH_2$ (600 mg) was dissolved in dry methanol (13 ml) and Ib_2O (1.3 g) added. The reaction was stirred at room temperature for 6 hours and water (20 ml) added. The mixture was stirred for a further 10 minutes and then filtered (Whatman № 1 filter paper) directly into acetone (200 ml). The resulting precipitate was collected by gravity filtration and washed with acetone (2 x 20 ml). The resultant solid was dried *in vacuo* to give both α-CDNIb and α-$CDNH_2$ (370 mg).

A portion of this material was passed through an Amberlite CG-120 (Type 1, 100 - 200 mesh) BDH ion exchange column, in its protonated form to remove α-$CDNH_2$ which was recovered by further elution with 4% ammonia solution. T.l.c. (solvent B) showed: R_C (relative to α-CD), α-CDNIb, 1.4. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-CDNIb, 0.45. FAB MS $M+H^+$ requires 1160, found 1160; $M+Na^+$ requires 1182, found 1182. Found: N 1.21. $C_{49}H_{77}NO_{30}$ requires: N 1.21%.

^1H n.m.r. and ^{13}C n.m.r. indicate an isomeric mixture of the α-CDNIb ^1H n.m.r. δ_H 0.87, J 7 Hz, Me_{iBu}, Me_{iBu}; 1.34, J 7 Hz, 1.39, J 7 Hz, αMe (2 isomers); 1.84, m, J 7 Hz, CH_{iBu}; 2.48, J 7Hz, $(CH_2)_{iBu}$; 3.2 -5.1, 60H; 7.23, q, ArH. ^{13}C n.m.r. (D_2O, dioxane standard) δ_C 18.5_0, q, 18.9_5, q, 23.3_0, q, 30.9_2, d, 41.2_4, t, 41.5_3, t, 45.7_8, t, 46.7_1, d, 47.1_6, d, 61.5_9, t, 71.7_4, 72.9_2, d, 73.2_8, d, 74.6_5, d, 82.2_0, d, 82.5_0, d, 84.0_2, 84.4_0, 102.7_0, d, 126.6_5, s, 128.2_8, d, 128.4_9, d, 130.6_8, d, 139.7_5, s, 140.0_0, s, 142.2_1, s.

EXAMPLE 44

Anhydrous α-$CDNH_2$ (600 mg) was dissolved in dry DMF (13 ml) and triethylamine (TEA, 0.3 ml) and the Ib_2O (Example 43, Step 1; 1.3 g) added. The reaction was stirred at room temperature for 6 hours and water (20 ml) added. The mixture was stirred for a further 10 minutes and then filtered (Whatman № 1 filter paper) directly into acetone (200 ml) . The resulting precipitate was left overnight to allow total precipitation, collected by gravity filtration (Whatman № 1 filter paper) and washed with acetone (2 x 20 ml). While still on the filter paper, the precipitate was dissolved in water and the resulting aqueous solution dried *in vacuo* to give a solid, shown by HPLC to be a mixture of α-CDNIb and α-$CDNH_2$ (370 mg) which may be purified as described in Example 43.

EXAMPLE 45

A solution of anhydrous α-$CDNH_2$ (0.195 g) and IbNP (Example 33, Step 1; 0.137 g) in dry pyridine (5 ml) was kept at room temperature overnight. The solution was evaporated under reduced pressure to leave a solid. This solid was triturated with ether (3 x 15 ml) to remove unreacted IbNP and other soluble materials. Water (2 ml) was added to the collected solid and evaporated under reduced pressure to remove any remaining pyridine. The solid was dried to give a yield of 0.23 g. This solid was dissolved in water (5 ml) and dropped into acetone (50 ml). The mixture was cooled in ice and the precipitate collected by filtration and rinsed with acetone (5 ml) and ether (5 ml). The solid was dissolved in water (5 ml), cyclohexane (0.3 ml) was added and the mixture stirred in an ice bath for 1 hour. The solid was separated in a centrifuge, rinsed with water (1 ml), acetone (1 ml) and ether (2 x 1 ml). The solid was suspended in water (2 ml) and heated to boiling. The solution evaporated under reduced pressure and dried to give α-CDNIb.

EXAMPLE 46

To a solution of α-methyl-4-(2-methylpropyl)-benzeneacetic acid (8.5 mg), ethyl chloroformate (4.5 mg) and TEA (4.2 mg) in

DMF (0.5 ml) was added α-CDNH$_2$ (10 mg) and the reaction mixture stirred at 80°C for 24 hours. Water was added followed by acetone to precipitate the product. The precipitate was separated by filtration through fluted № 50 qualitative Whatman filter paper, washed with acetone and the solid collected and dried *in vacuo* to give a material with the same HPLC characteristics as α-CDNIb.

EXAMPLE 47

6^A-Amino-6^A-deoxy-6^A-N-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-β-cyclodextrin (β-CDNIb)

A mixture of β-CDNH$_2$ (845 mg) and Ib$_2$O (Example 43, Step 1; 1700 mg) were dissolved in methanol (20 ml) and refluxed overnight. After cooling, the mixture was poured into acetone (100 ml). The resulting precipitate was collected by gravity filtration (Whatman № 1 filter paper), washed with acetone and recrystallized from water to give β-CDNIb (522 mg). T.l.c. (solvent B) showed: R_C (Relative to β-CD), β-CDNIb, 1.3. HPLC using 70% CH_3CN-H_2O as eluant showed: t_R (Relative to β-CD), β-CDNHIb, 0.27.

$\upsilon_{c=o}$ 1651 cm^{-1}. Found: N 1.09. $C_{55}H_{87}NO_{35}$ requires: N 1.06%. FAB MS M+H$^+$ requires 1322 found 1322; M+Na$^+$ requires 1344 found 1344. ^{1}H n.m.r. (D_6DMSO) δ_H 0.9 - 2.6, 12H; 3.0 - 6.1, 70H; 7.0 - 7.4, 4H. ^{13}C n.m.r. (D_6DMSO) δ_C 22.4_8, 22.7_3, 26.1_4, 33.5_4, 48.2_2, 48.5_1, 63.8_0, 85.3_4, 85.5_4, 87.2_7, 87.6_0, 105.9_5, 131.0_0, 132.6_2, 143.0_9, 143.4_1, 143.6_3, 177.7_1, 177.9_2.

EXAMPLE 48

6^A-Amino-6^A-deoxy-6^A-N-((S)-6-methoxy-α-methyl-2-naphthaleneacetyl)-α-cyclodextrin (α-CDNNp)

Step 1: (S)-6-Methoxy-α-methyl-2-naphthaleneacetic acid anhydride (Np$_2$O) was prepared as follows. (S)-6-Methoxy-α-methyl-2-naphthaleneacetic acid (250 mg) was dissolved in dry ether (10 ml) and DCC (130 mg) was added. Insoluble DCU formed immediately. After 1 hour the ether was removed *in vacuo* and ethyl acetate (100 ml) added to the residue. The product only

slowly dissolved in ethyl acetate so sufficient agitation and time was required to ensure solubilization. The insoluble DCU was filtered off and ethyl acetate removed *in vacuo* to yield Np_2O. υ_{max} 1741, 1806 cm^{-1}. ^1H n.m.r. ($CDCl_3$) δ_H 1.2, d, 3H; 3.8, q, 1H; 3.95, s, 3H; 7.3, m, 6H.

Step 2: α-$CDNH_2$ (28 mg) was dissolved in dry methanol (0.65 ml) and Np_2O (60 mg) added. The reaction was stirred at room temperature overnight. Acetone was added until precipitation appeared complete. The resulting precipitate was collected by gravity filtration (Whatman № 1 filter paper) and washed with acetone. The solid was collected and dried *in vacuo*. HPLC analysis showed two major and two minor products. The dried crude product was dissolved in 70% aqueous CH_3CN (5 ml) and chromatographed on Sephadex eluting with the same solvent. α-CDNNp was isolated as a colorless solid (190 mg). T.l.c. showed: R_C (relative to α-CD), α-CDNNp, 1.4. HPLC using 70% CH_3CN-H_2O showed: t_R (relative to α-CD), α-CDNNp, 0.43.

$\upsilon_{c=o}$ 1641, 1607 cm^{-1}. Found: N 1.11. $C_{50}H_{78}NO_{31}$ requires: N 1.18%. FAB MS $M+H^+$ requires 1184 found 1184; $M+Na^+$ requires 1206 found 1206. ^1H n.m.r. (D_6DMSO) δ_H 1.3 - 1.4, d, Me; 3.1 - 5.8, 60H; 7.1 - 8.2, Naphthalene hydrogens. ^{13}C n.m.r. (D_6DMSO) δ_C 22.5_5, 22.7_9, 48.5_6, 59.1_0, 63.9_9, 74.0_0, 76.0_8, 77.0_2, 77.2_2, 86.0_2, 87.8_0, 105.4_2, 105.9_0, 106.1_2, 109.6_6, 122.4_4, 129.15, 130.3_8, 132.3_3, 133.0_1, 136.9_9, 141.5_8, 148.4_5, 177.6_9, 188.0_4.

EXAMPLE 49

6^A-Amino-6^A-deoxy-6^A-N-((S)-6-methoxy-α-methyl-2-naphthaleneacetyl)-β-cyclodextrin (β-CDNNp)

β$CDNH_2$ (800 mg) and Np_2O (Example 48, Step 1; 1600 mg) were dissolved in a mixture of methanol and DMF (3:1; 16 ml) and refluxed overnight. The methanol was then removed *in vacuo* and the residue poured into acetone (60 ml). The resulting precipitate was collected by gravity filtration (Whatman № 1 filter paper), washed with acetone and recrystallized from water to give β-CDNNp (525 mg). T.l.c. (solvent B) showed: R_C

(Relative to β-CD), β-CDNNp, 1.5. HPLC using 70% CH_3CN-H_2O as eluant showed: t_R (Relative to β-CD), β-CDNNp, 0.35.

$\upsilon_{c=o}$ 1608, 1629 cm^{-1}. Found: N 1.09. $C_{56}H_{83}NO_{36}$ requires: N 1.04%. FAB MS $M+H^+$ requires 1346, found 1346. ^1H n.m.r. (D_6DMSO) δ_H 1.4 - 1.6, Me; 3.0 - 6.1, 69H; 7.1 - 8.2, ArH. ^{13}C n.m.r. (D_6DMSO) δ_C 22.7_0, 28.4_3, 29.2_7, 37.2_9, 48.6_1, 51.5_2, 59.0_9, 63.8_2, 73.9_1, 75.9_9, 76.4_2, 77.0_0, 85.4_5, 87.6_1, 105.5_7, 105.9_4, 109.6_7, 122.4_3, 129.1_4, 130.3_9, 132.3_3, 133.0_2, 136.9_9, 141.5_3, 160.9_2, 177.6_8.

EXAMPLE 50

(2^S,3^S)-3^A-Amino-3^A-deoxy-3^A-((S)-6-methoxy-α-methyl-2-naphthaleneacetyl)-α-cyclodextrin (α-3CDNNp)

A solution of α-3CDNH2 (1.6 g) and Np_2O (Example 48, Step 1, 3.2 g) in dry dimethylformamide (20 ml) was stirred at 60° overnight. The solution was concentrated *in vacuo* to 2 ml and then poured into acetone (50 ml). The precipitate was collected by filtration, rinsed with acetone. The solid was passed through a Sphadex G15 column to give 300 mg of yellow solid. T.l.c. (solvent A) of the product showed: R_C (relative to α-CD), 1.43. HPLC using 70% acetonitrile in water as eluant showed t_R (relative to α-CD) 0.28

υ_{max} (KBr disk) 1637, 1532 cm^{-1}. FAB MS $M+H^+$requires 1184 found 1184; $M+Na^+$ requires 1206 found 1206. ^1H n.m.r (d_6DMSO) δ_H 1.5(d); 3.5 - 5.5; 7.2 - 8.3. ^{13}C n.m.r. (D_2O) 19.3_2, 51.9_8, 56.6_9, 61.7_4, 67.8_2, 72.4_6, 72.6_4, 72.9_4, 73.2_2, 74.3_1, 74.6_5, 81.7_4, 82.3_2, 82.7_5, 82.9_3, 102.3_2, 103.1_0, 107.6_7, 119.8_7, 121.9_0, 127.0_6, 129.1_5, 130.0_5, 130.9_4, 131.8_0, 133.3_2, 134.7_6, 137.2_6.

The preparations of 6^A-Amino-6^A-N-(4-aminobutyl)-6^A-deoxy-α-cyclodextrin (α-CDN4N) described in Examples 51 and 52 are representative of the procedures used in Examples 51 to 55

EXAMPLE 51

6^-Amino-6^-N-(4-aminobutyl)-6^-deoxy-α-cyclodextrin (α-CDN4N)

α-CDOTs (86 mg) and 1,4-diaminobutane (0.3 ml) were heated together at 70°C for 3 hours. T.l.c. (solvent A) indicated that there was no α-CDOTs left. Acetone (4 ml) was added and the resulting suspension centrifuged. Following removal of the supernatant, the solid was dissolved in water (0.1 ml), precipitated with acetone (4 ml) and centrifuged. The solid was collected and dried in a vacuum desiccator over phosphorus pentoxide, to yield an off-white powder (84 mg) which still smelt of diaminobutane. The crude product was dissolved in boiling water (1.0 ml) and filtered hot. Ethanol was added to the boiling solution until turbidity was retained, followed by the addition of water until the solution remained clear. Upon cooling a white solid formed which was recrystallized from hot water to give pure α-CDN4N. An alternative method of purification is Soxhlet extraction with ethanol to remove diaminobutane, followed by precipitation from a water cyclohexane emulsion. The solid is collected and recrystallized from water.

FAB MS $M+H^+$ requires 1043, found 1043. ^1H n.m.r. (D_6DMSO) δ_H 1.3 - 3.0, 10H; 3.1 - 4.9, 60H. ^{13}C n.m.r. (D_2O, dioxane std) δ_C 27.2_4, 29.6_0, 41.4_7, 49.8_6, 50.6_5, 61.5_9, 71.8_7, 72.9_7, 73.3_5, 74.6_6, 82.4_3, 85.0_0, 102.8_1.

EXAMPLE 52

α-CDOTs (30 g) was dissolved in DMF (50 ml) with diaminobutane (5 g) and heated at 100°C for 3 hours. The mixture was poured into acetone (200 ml) and the solid was filtered off. Then the solid was dissolved in hot water (50 ml) and left to recrystallize. After standing for 2 days the solid was filtered off. Soxhlet extraction with ethanol overnight removed trapped diaminobutane. Recrystallization from hot water afforded pure α-CDN4N after prolonged standing.

EXAMPLE 53

6^A-Amino-6^A-N-(3-aminopropyl)-6^A-deoxy-β-cyclodextrin (β-CDN3N)

FAB MS M+H$^+$ requires 1191, found 1191. ^{1}H n.m.r. (D_6DMSO) δ_H 1.5 - 1.7, 2H; 2.5 - 3.0, 4H; 3.2 - 6.1, 69H. ^{13}C n.m.r. (D_2O, dioxane std) δ_C 29.8_9, 39.2_7, 47.2_9, 50.3_3, 61.2_8, 71.3_5, 72.8_1, 73.0_5, 74.0_7, 82.1_0, 84.57, 102.4_9, 102.8_0.

EXAMPLE 54

6^A-Amino-6^A-N-(4-aminobutyl)-6^A-deoxy-β-cyclodextrin (β-CDN4N)

Precipitation was effected by the addition of ethanol rather than acetone. The solid was filtered off and Soxhlet extraction with ethanol removed most of the trapped diaminobutane. Recrystallization of the solid twice from hot water afforded β-CDN4N containing very large quantities of solvent, and loses up to 40% of it's weight upon drying.

FAB MS M+H$^+$ requires 1205, found 1205. ^{1}H n.m.r. (D_6DMSO) δ_H 1.3 - 1.5, 4H; 2.4 - 3.0, 6H; 3.1 - 4.9, 59H. ^{13}C n.m.r. (D_2O, dioxane std) δ_C 26.6_5, 28.9_3, 41.0_8, 49.4_2, 50.3_3, 61.3_2, 71.2_9, 72.9_1, 73.0_4, 74.1_2, 81.7_9, 82.1_6, 84.4_9, 102.3_6, 102.8_3.

EXAMPLE 55

6^A-Amino-6^A-N-(6-aminohexyl)-6^A-deoxy-β-cyclodextrin (β-CDN6N)

FAB MS M+H$^+$ requires 1233, found 1233. ^{1}H n.m.r. (D_6DMSO) δ_H 1.2 - 3.0, 14H; 3.1 - 6.0, 70H. ^{13}C n.m.r. (D_2O; dioxane std) δ_C 26.7_9, 27.1_7, 28.6_5, 30.6_0, 41.3_4, 48.3_2, 49.4_5, 61.2, 70.1_9, 73.1_6, 74.3_8, 81.2_6, 82.2_8, 84.0_6, 101.9_1, 103.0.

EXAMPLE 56

Bis-($3^A(2^A)$-deoxy-$3^A(2^A)$-α-cyclodextrin) succinamide (α-$3CD_2NSc$)

Step 1: Bis-(3-nitrophenyl) succinate can be prepared as follows. Succinic acid (11.8 g) and 3-nitrophenol (27.8 g) were dissolved in dry ethyl acetate (1 l, 4Å sieve) and the solution

stirred at 0°C. DCC (40 g) was added and the resultant solution stirred for 1 hour at 0°C then at room temperature overnight. The reaction mixture was vacuum filtered (Whatman № 1 paper) and the collected DCU washed with ethyl acetate (50 ml). The bright yellow filtrate was concentrated *in vacuo* to about 300 ml . Material which had come out of solution was redissolved by boiling the solution. The hot solution was filtered (gravity, Whatman № 1 paper) and allowed to cool slowly. The crystallized bis-(3-nitrophenyl) succinate was collected by vacuum filtration, rinsed with cold ethyl acetate (20 ml) and dried *in vacuo* to give bis-(3-nitrophenyl) succinate (21.34 g). The crude solid can alternately be purified by silica gel chromatography. M.p. 153-154°C. υ_{max} 1752 cm^{-1}. ^1H n.m.r. ($CDCl_3$) δ_H 3.1, s, 4H; 7.2 - 8.6, ArH.

Step 2: Bis-(3-nitrophenyl) succinate (20 mg) was added in one portion to a stirred solution of α-3(2)$CDNH_2$ (100 mg) in DMF (3 ml). The solution was left to stir at room temperature for two weeks, until t.l.c. showed no further reaction. The solution was poured into acetone (30 ml) and the resultant precipitate collected by filtration. The solid was rinsed with acetone and ether and air dried to give the crude product (93 mg). T.l.c. (solvent B) of the product showed: R_C (relative to α-CD), α-3CD_2NSc, 0.75. HPLC of the product using a 60% acetonitrile - water eluant showed: t_R (relative to α-CD), α-3CD_2NSc, 2.68. FAB MS $M+H^+$ requires 2026, found 2026.

EXAMPLE 57

Bis-(6^A-deoxy-6^A-β-cyclodextrin) terephthalate (β-CD_2T_p)

A mixture of β-CDOTs (1.29 g) and dicesium terephthalate (0.215 g) was heated in dry DMF (5 ml) at 100 - 110°C for 18 hours. The reaction mixture was cooled and acetone was added until precipitation was complete. The precipitate was collected by vacuum filtration, washed with acetone and recrystallized from water to furnish a white solid (0.8 g). HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), 0.54, 0.77, 1.0, 2.90, 3.57 (major). Preparative HPLC of the crude mixture allowed the

isolation of the major product (t_R 3.57). FAB MS M+H$^+$ requires 2399 found 2399; M+Cs$^+$ requires 2532 found 2532 .

EXAMPLE 58

N,N'-Bis-(6^A-deoxy-6^A-β-cyclodextrin) oxalamide (β-CD$_2$NOx)

Step 1: Bis-(3-nitrophenyl) oxalate can be prepared as follows. To a solution of 3-nitrophenol (2 g) in dry ether (36 ml) under an atmosphere of nitrogen was added triethylamine (1.46 g, 14.4 mmol). Oxaloyl chloride (2.01 g) was added to the bright yellow solution over a period of 5 minutes resulting in the formation of a white slurry. The slurry was shaken periodically over 3 hours. The solvent was removed *in vacuo* and the resulting white solid was stirred in chloroform (100 ml) for 5 minutes to remove any triethylamine hydrochloride. The remaining solid was collected by vacuum filtration and washed with chloroform (2 x 10 ml) and ether (5 ml), and dried in a desiccator *in vacuo* . The crude product was recrystallized from nitrobenzene taking care not to allow the solvent to reach 100°C. After 24 hours the white powder was collected by vacuum filtration, washed with ether (2 x 5 ml) and dried in a desiccator *in vacuo* to give bis-(3-nitrophenyl) oxalate as a white powder (1.32 g). M.p. 229-230°C. υ_{max} 1767, 1520 cm^{-1}.

Step 2: To a stirred solution of dry β-CDNH$_2$ (250 mg, 0.220mmol) in dry pyridine (5 ml) was added bis (3-nitrophenyl) oxalate (38 mg, 0.114 mmol) over a period of 1 hour. The reaction mixture was left to stir at room temperature for 18 hours and then the solution was evaporated to dryness. Residual pyridine was removed from the product by co-distillation with water *in vacuo*. The resulting solid was dissolved in water (5 ml), filtered and then acetone was added to the stirred filtrate to give a white solid which was collected by vacuum filtration and washed with acetone (2 x 20 ml). This precipitation procedure was repeated and the product dried to constant weight over P_2O_5 to give the pure dimer (235 mg, 92%). Tlc (solvent B) of the product showed: R_C (relative to βCD), 0.4. HPLC of the product using a 60% acetonitrile-water eluant showed: t_R

(relative to βCD), 3.5.

υ_{max} 1675, 1653, 1559 cm^{-1}. ^{13}C nmr (D_2O) δ 41.0_9, 61.2_5, 70.9_8, 72.9_7, 73.7_6, 74.0_5, 82.0_6, 83.8_5, 102.8_1, 161.7_9 FABMS $M+H^+$ requires 2322 found 2322; $M+Na^+$ requires 2344 found 2344. Anal. calcd. for $C_{86}H_{140}N_2O_{70}$: C, 44.84; H, 6.07; N, 1.20 found: C, 42.09; H, 6.13; N, 1.25. $C_{86}H_{140}N_2O_{70}.7H_2O$ requires: C, 42.19; H, 6.34; N, 1.14.

EXAMPLE 59

N,N'-Bis-(6^A-deoxy-6^A-α-cyclodextrin) succinamide (α-CD_2NSc)

Step 1: Succinic acid (5.9 g, 0.05 mol) and 3-nitrophenol (13.9 g, 0.1 mol) were dissolved in dry DMF (300 ml) and the solution allowed to stir at room temperature for 15 minutes. DCC (20 g, 0.1 mol) in dry DMF (50 ml) was added and the solution allowed to stir at room temperature for 48 hours during which time the solution became a bright opaque yellow. The resultant DCU was removed by vacuum filtration through a sintered glass funnel, leaving the filtrate as a bright clear yellow solution. The DMF was removed *in vacuo* to leave a pale yellow solid which was recrystallized from dry ethyl acetate (200 ml), during which DCU was removed by hot filtration. After standing for 90 minutes the remaining DCU crystallized out as fine off-white needles and collected by gravity filtration (Whatman № 1 filter paper). The filtrate was placed in a fridge at 4°C overnight and bis-(3-nitrophenyl) succinate collected by vacuum filtration as a fine white powder (6.5 g). M.p. 153-154°C.

Step 2: Bis-(3-nitrophenyl) succinate (160 mg) was added in portions to a solution of α-$CDNH_2$ (1 g) in dry pyridine with stirring over one hour. The reaction was followed by t.l.c. and appeared to be complete after 5 days. The pyridine was removed *in vacuo* and the solid dissolved in water. The water was removed *in vacuo* to remove traces of pyridine. The solid was dissolved in a minimum of water (8 ml) and added dropwise to ice-cold acetone (80 ml) and stirred rapidly for 10 minutes. The white powdery solid was collected by vacuum filtration and washed with

ice-cold acetone (2 x 3 ml) and ice-cold ether (2 x 3 ml). The solid was dissolved in water (10 ml) and cyclohexane (0.5 ml) added then the solution stirred vigorously in an ice-bath for 1.5 hours. The precipitate was collected by centrifugation and washed with ice-cold water (10 ml), ice-cold acetone (2 x 10 ml) and ice-cold ether (2 x 10 ml). The white precipitate was suspended in water (50 ml) and boiled until it dissolved. The water was removed *in vacuo* and the solid redissolved in a minimum of water, filtered, and added to ice-cold acetone (150 ml). The white precipitate was collected by vacuum filtration and washed with acetone and ether to give α-CD_2NSc (855 mg). T.l.c. showed: R_C (relative to α-CD), α-CD_2NSc, 0.375. HPLC using a 65% CH_3CN-H_2O eluant showed: t_R (relative to α-CD), α-CD_2NSc, 3.2.

υ_{max} 1558 cm^{-1}. FAB MS $M+H^+$ requires 2027, found 2027. ^{13}C n.m.r. (D_2O) δ_C 32.1_6, t; 41.3_9, t; 61.2_5, t; 61.6_4, t; 71.5_3, d; 72.8_9, d; 73.0_3, d; 73.2_1, d; 74.2_8, d; 74.5_3, d; 82.3_9, d; 84.3_0, d; $102._6$, d; 175.8_5, s.

Examples 60 and 61 illustrate alternative methods for the preparation of N,N'-Bis-(6^A-deoxy-6^A-β-cyclodextrin) - succinamide.

EXAMPLE 60

N,N'-Bis-(6^A-deoxy-6^A-β-cyclodextrin) succinamide (β-CD_2NSc)

To a solution of β-$CDNH_2$ (310 mg) in DMF (5 ml) was added bis-(3-nitrophenyl) succinate (Example 59, Step 1; 48 mg) in small portions (ca. 5 $mg.hour^{-1}$). [note: all the β-$CDNH_2$ might not be in solution but after the first few additions of the diester a clear yellow solution should be obtained. Alternatively, the addition of 1.22 ml of dry TEA causes dissolution of the β-$CDNH_2$ almost immediately]. The solution was then stirred at room temperature (ca. 15°C) for 7 days, t.l.c. shows the reaction progresses slowly. The solvent was removed *in vacuo* (oil pump) to ca. 1 ml and acetone (10 ml) was added to give a precipitate. The mixture was stirred rapidly for 10 minutes and collected by vacuum filtration. The solid was washed

with acetone (2 x 5 ml), then with diethyl ether (2 x 5 ml), and air dried to give 350 mg of a crude off-white solid. This solid was dissolved in a minimum volume of water (ca. 3 ml) to give a yellow solution which was then filtered through cotton wool. Cyclohexane (0.25 ml) was added and the mixture rapidly stirred for 1 hour to yield a fine white solid in suspension. The mixture was centrifuged, the supernatant decanted off, the solid washed with cold water (ca. 1 ml), centrifuged again, decanted and the procedure repeated twice with acetone. The solid was dried *in vacuo* over P_2O_5 to give 220 mg of a white solid. The solid was suspended in water (15 ml) and dissolved with heating and heated on a water bath to drive off cyclohexane. Removal of water under reduced pressure produces a glassy solid difficult to remove from the sides of the vessel. This solid may be recovered by dissolution in a minimum volume of water and subsequent precipitation with acetone and collection by filtration and washing with acetone and ether. Drying of the solid *in vacuo* gives a white solid (210 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CD_2NSc, 0.33. HPLC using a 75% acetonitrile - water eluant showed: t_R (relative to β-CD), β-CD_2NSc, 5.6.

υ_{max} 1642, 1559 cm^{-1}. FAB MS $M+H^+$ requires 2350 found 2350. ^{13}C n.m.r. (D_2O) δ_C 31.9_3, 41.0_1, 61.2_0, 71.1_6, 72.7_6, 72.9_7, 74.0_1, 82.0_3, 83.9_7, 175.5_3.

EXAMPLE 61

Bis-(3-nitrophenyl) succinate (Example 59, Step 1; 48 mg) was added in portions over an hour to a stirred solution of dry (β-$CDNH_2$) (310 mg) in dry DMF (5 ml, 4Å sieve). The reaction mixture was stirred at room temperature for 7 days by which time t.l.c. showed that no β-$CDNH_2$ remained in the reaction mixture. The solution was concentrated *in vacuo* to ~1 ml and acetone (10 ml) was added with vigorous stirring. The resulting off-white solid was collected by filtration (Whatman № 1 paper), washed with acetone (2 x 5 ml), ether (2 x 5 ml) and air dried to give 350 mg of crude product. This material was dissolved in water

(5 ml), filtered and cyclohexane (0.25 ml; spectroscopic grade) added. The mixture was stirred vigorously in an ice-bath for 1 hour to give a fine white solid which was collected by centrifugation (3000 r.p.m., 10 minutes). The solid was washed successively with ice cold water (1 ml), acetone (2 x 5 ml) and ether (2 x 5 ml). The air dried solid was resuspended in water (15 ml) and heated on a boiling water bath for 30 minutes. After cooling to room temperature the solution was dried *in vacuo* to give a glassy solid which was dissolved in water (1 ml) and precipitated with acetone (20 ml). The precipitate was collected by vacuum filtration, rinsed with acetone (2 x 1 ml) and dried to give 210 mg of white powder. Drying over P_2O_5 *in vacuo* gave β-CD_2NSc (205 mg).

EXAMPLE 62

Purification of β-CD_2NSc on BioRex 70

The acid form of BioRex 70 was prepared by taking BioRex 70 (Na^+ form, as supplied, 100 ml) and batch rinsing in a scintered glass funnel as follows:-
Water (10 x 100 ml), 0.5 M sodium hydroxide (5 x 100 ml), water (10 x 100 ml), 10% hydrochloric acid (5 x 100 ml), water (10 x 100 ml) and Milli-Q® water (10 x 100 ml). This procedure gives 50 - 60 ml of the resin in the protonated form.

The resin from above (~ 50 ml) was suspended in Milli-Q® water (200 ml) with stirring. A solution of β-CD_2NSc (8 g) in Milli-Q® water (100 ml) was added and the mixture left to stir overnight at room temperature. The resin was collected by filtration in a scintered glass funnel and rinsed with water (5 x 100 ml) at 50°C. The combined filtrates were dried *in vacuo* to give 4.7 g of β-CD_2NSc. This was dissolved in water (20 ml) and filtered (0.22 μ filter). Ethanol (~ 20 ml) was added until a faint haze persisted. The haze was cleared with gentle heating and the flask containg the dissolved β-CD_2NSc placed in a sealed vessel with a reservoir of ethanol (~ 100 ml). When crystallization was complete the product was collected by filtration, washed with ethanol (10 ml), air dried and finally

dried *in vacuo* to give pure β-CD_2NSc (3.8 g).

The β-$CDNH_2$ bound to the resin was isolated by rinsing the resin with a 20% ammonia solution (5 x 100 ml) warmed to 50°C. The filtrate was dried *in vacuo* to give 2.9 g of amine. HPLC analysis indicated there were two unidentified materials present with the amine. HPLC of the product using a 60% acetonitrile - water eluant showed: t_R (relative to β-CD), 1.0, 2.43, 2.65, 3.06.

EXAMPLE 63

N,N'-Bis(6^A-deoxy-6^A-β-cyclodextrin) glutaramide (β-CD_2NGl)

Step 1: To a solution of glutaric acid (10 g) and 3-nitrophenol (21 g) in ethyl acetate (750 ml) cooled to 0° was added DCC (30 g). The resultant solution was stirred overnight at room temperature. The mixture was filtered to remove DCU and the filtrate was evaporated to dryness *in vacuo* to give a yellow solid. This was dissolved in boiling ethyl acetate (300 ml) and undissolved solid was removed by filtration. The crystallized product was collected by vacuum filtration, rinsed with cold ethyl acetate and dried to give bis-(3-nitrophenyl) glutarate as a cream coloured powder (10.81 g). ν_{max} 1758 cm^{-1}.

Step 2: To a solution of β-$CDNH_2$ (250 mg) in pyridine (3 ml) was added bis-(m-nitrophenyl) glutarate (45 mg) in small portions over 5 hours. The solution was stirred at room temperature for 5 days after which time t.l.c. showed one major new product and a small amount of β-$CDNH_2$. The solution was evaporated to dryness and residual pyridine was removed by co-distillation with water *in vacuo*. The white solid was dissolved in water (10 ml) and this solution was added to a suspension of Bio Rex 70 (H^+ form, 1 g) also in water (20 ml). After stirring at room temperature for 18 hours the mixture was filtered (0.22 μm) and the filtrate passed through a squat column of Sephadex G15. This solution was evaporated *in vacuo* to ~1 ml and addition of acetone (30 ml) gave a white precipitate which was collected by vacuum filtration, washed with acetone (2 x 20 ml) and dried

in vacuo to give β-CD_2NGl (156 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CD_2NGl, 0.30. HPLC using a 65% acetonitrile - water eluant showed: t_R (relative to β-CD), β-CD_2NGl, 3.4.

υ_{max} 1642, 1551 cm^{-1}. ^{13}C n.m.r. (D_2O) δ_C 22.6_5, t; 35.9_0, t; 41.0_3, t; 61.0_1, t; 61.2_6, t; 71.1_6, d; 72.8_1, d; 73.0_2, d; 74.0_7, d; 82.0_4, d; 84.1_1, d; 102.8_0, d; 176.6_6, s. FAB MS $M+H^+$ requires 2364, found 2364; $M+Na^+$ requires 2386, found 2386.

EXAMPLE 64

6^-Amino-6^-deoxy-6^-N-(4-O-(3-nitrophenyl)-carboxypropanoyl)-α-cyclodextrin (α-CDNScNP)

To a solution of bis-(3-nitrophenyl) succinate (Example 59, Step 1, 18.52 g) in DMF (350 ml) was added α-$CDNH_2$ (5 g), in portions, over 3 hours. The reaction mixture was left to stir at room temperature overnight. The solution was concentrated *in vacuo* to ca. 15 ml and the residue was triturated with ice-cold acetone (450 ml). The precipitated solid was collected by vacuum filtration, rinsed with acetone and dried under vacuum to give a white powder (10.1 g). T.l.c. (solvent B) of the product showed: R_C (relative to α-CD), 1.61

υ_{max} 1752 cm^{-1}. FAB MS $M+Na^+$ requires 1216, found 1216.

EXAMPLE 65

6^-Amino-6^-deoxy-6^-N-(4-O-(3-nitrophenyl)-carboxypropanoyl)-β-cyclodextrin (β-CDNScNP)

To a solution of bis-(3-nitrophenyl) succinate (Example 59, Step 1; 500 mg) in DMF (10 ml) was added β-$CDNH_2$ (300 mg) in small portions over a 24 hour period. Stirring at room temperature was continued for 18 hours after the final addition of β-$CDNH_2$. Evaporation *in vacuo* to ca. 1 ml and addition of acetone (30 ml) with stirring gave an off white solid which was collected by vacuum filtration and washed with acetone (2 x 20 ml) and diethyl ether (2 x 5 ml). Drying to constant weight over P_2O_5 *in vacuo* gave β-CDNScNP (340 mg). T.l.c. (solvent B) of the

product showed: R_C (relative to β-CD), β-CDNScNP, 1.3.

υ_{max} 1770, 1652, 1533 cm^{-1}. Found: N 2.13. $C_{52}H_{78}N_2O_{39}$ requires: N 2.07%. FAB MS M+H^+ requires 1355 found 1355. ^{13}C n.m.r. (D_6DMSO) δ_C 33.2_1, 33.6_4, 63.8_9, 73.8_0, 76.0_0, 76.3_9, 76.9_7, 85.3_7, 85.5_7, 87.5_0, 105.9_3, 121.1_5, 124.6_5, 132.8_2, 134.7_2, 152.1_9, 154.8_3, 174.7_7, 175.1_0.

EXAMPLE 66

N-(6^A-deoxy-6^A-α-cyclodextrin)-N'-(6^A-deoxy-6^A-β-cyclodextrin) succinamide (α,β-CD_2NSc)

A mixture of α-$CDNH_2$ (100 mg) and β-CDNScNP (Example 65; 100 mg) was stirred at room temperature in DMF (2 ml) for 24 hours. After this time t.l.c. showed: R_C (relative to β-CD) 0.3, α,β-CD_2NSc; 0.8, α-$CDNH_2$ (trace). Evaporation to ca. 0.5 ml and addition of acetone (20 ml) gave an off white powder which was collected by vacuum filtration and washed with acetone (2 x 10 ml) and ether (2 x 5 ml) to give 139 mg of crude product. The solid was dissolved in water (10 ml) and stirred for 18 hours with Bio-Rex® 70 (H^+ form, 2 g, 2.4 meq.ml^{-1}) also in water (10 ml). Filtration and evaporation of the filtrate to ca. 1 ml followed by the addition of cyclohexane (0.25 ml) with rapid stirring yielded a fine white solid in suspension. The mixture was centrifuged, the supernatant removed by decantation, the solid washed with cold water (ca. 1 ml), centrifuged again, decanted and the procedure repeated twice with acetone. The solid was suspended in water (15 ml) and dissolved with heating on a boiling water bath until all of the cyclohexane had been driven off. Removal of the water *in vacuo* to ca. 0.5 ml followed by addition of acetone (20 ml) afforded a white solid which was collected by filtration, washed with acetone (2 x 20 ml) and dried *in vacuo* over P_2O_5 to in a yield of 125 mg. T.l.c. (solvent B) showed: R_C (relative to β-CD), α,β-CD_2NSc, 0.30. HPLC using a 65% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), α,β-CD_2NSc, 4.9.

υ_{max} 1558, 1645 cm^{-1}. Found: N 1.23. $C_{82}H_{134}N_2O_{65}$ requires: N

1.28%. FAB MS M+H$^+$ requires 2188, found 2188. ^{13}C n.m.r. (D_2O) δ_C 32.0, 41.1$_9$, 61.0$_9$, 61.3$_0$, 61.4$_7$, 71.3$_0$, 72.7$_2$, 72.8$_5$, 73.0$_4$, 74.1$_3$, 74.3$_3$, 82.1$_7$, 84.1$_0$, 102.4$_0$, 102.8$_7$, 175.6$_6$.

Examples 67 and 68 illustrate alternative methods for the preparation of 6^A-Amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-β-cyclodextrin.

EXAMPLE 67

6^A-Amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-β-cyclodextrin (β-CDNSc)

A mixture of β-CDNH$_2$ (500 mg) and succinic anhydride (51 mg) in DMF (5 ml) was stirred at room temperature for 24 hours after which time t.l.c. indicated that all starting materials had been consumed. Evaporation of the solvent to a glassy oil followed by the addition of acetone (20 ml) with trituration gave a white solid which was collected by vacuum filtration and washed with acetone (2 x 20 ml). The dried solid (600 mg) was dissolved in a minimum amount of water (ca. 2 - 3 ml) and filtered through a 0.2 μm filter (the filter was then rinsed with 1 ml of water). Evaporation of the combined filtrates to ca. 1 ml and the addition of acetone (30 ml) with stirring gave a white solid which was collected by vacuum filtration and washed with acetone (2 x 5 ml). Drying to constant weight over P_2O_5 *in vacuo* gave β-CDNSc (470 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CDNSc, 1.1. HPLC using 60% CH_3CH-H_2O + SAM eluant showed: t_R (relative to α-CD), β-CDNSc, 1.88.

υ_{max} 1721, 1652, 1558 cm^{-1}. FAB MS M+H$^+$ requires 1234 found 1234. ^{13}C n.m.r. (D_2O) δ_C 30.2$_7$, 31.1$_6$, 41.1$_6$, 61.0$_1$, 61.2$_2$, 71.6$_6$, 72.7$_7$, 73.0$_8$, 74.0$_9$, 81.6$_6$, 82.1$_2$, 84.1$_1$, 102.5$_4$, 102.9$_2$, 175.5$_8$, 177.5$_2$.

EXAMPLE 68

A solution of β-CDNScNP (Example 65; 50 mg) in water (20 ml) was stirred at reflux for 5 hours. Evaporation *in vacuo* to ca. 1 ml followed by the addition of acetone (20 ml) with stirring

gave a white solid which was collected by vacuum filtration and washed with acetone (2 x 10 ml). Drying to constant weight over P_2O_5 *in vacuo* gave β-CDNSc (30 mg) which was identified by comparison of its physical data with that of an authentic sample prepared previously.

EXAMPLE 69

6^-Amino-6^-deoxy-6^-N-(4-carboxybutanoyl)-β-cyclodextrin (β-CDNGl)

A mixture of β-$CDNH_2$ (500 mg) and glutaric anhydride (75 mg) in DMF (7 ml) was stirred at room temperature for 18 hours. Evaporation of the solvent to a glassy oil followed by the addition of acetone (30 ml) with trituration gave a white solid which was collected by vacuum filtration and washed with acetone (2 x 20 ml). The dried solid was dissolved in a minimum amount of water (ca. 5 ml) and filtered through a 0.2 μm filter (the filter was then rinsed with water 1 ml). Evaporation of the combined filtrates to ca. 1 ml and the addition of acetone (30 ml) with stirring gave a white solid which was collected by vacuum filtration and washed with acetone (2 x 5 ml). Drying to constant weight over P_2O_5 *in vacuo* gave β-CDNGl (550 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CDNGl, 1.1.

$\upsilon_{c=o}$ 1713, 1657 cm^{-1}. Found: N 1.11. $C_{42}H_{77}NO_{37}$ requires: N 1.12%. FAB MS $M+H^+$ requires 1248 found 1248. ^{13}C n.m.r. (D_2O) δ_C 21.6_0, 34.1_6, 35.7_8, 41.2_1, 60.9_5, 61.2_7, 71.6_1, 73.0_6, 74.2_8, 81.9_6, 82.1_7, 82.2_7, 84.4_0, 102.8_2, 103.0_7, 103.2_5, 176.6_2, 178.6_6.

EXAMPLE 70

6^-Amino-6^-deoxy-N-(3-aminocarbonylpropanoyl)-β-cyclodextrin (β-CDNScN)

A solution of β-CDNScNP (Example 65; 250 mg) in concentrated ammonia (20 ml, 28%) was stirred at room temperature for 18 hours. The solution was filtered (Whatman № 1 filter paper) and the paper washed with water (5 ml). The combined filtrates were evaporated to ca. 1 ml and the addition of acetone (30 ml) with stirring gave a white solid which was collected by vacuum

filtration and washed with acetone (2 x 10 ml) and ether (2 x 5 ml). Drying to constant weight over P_2O_5 *in vacuo* gave β-CDNScN (215 mg). This solid was recrystallized from water (acetone vapour diffusion) to give colourless microcrystals of β-CDNScN (200 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CDNScN, 1.2. HPLC using a 70% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), β-CDNScN, 1.04.

υ_{max} 1653, 1559 cm^{-1}. FAB MS $M+H^+$ requires 1233 found 1233. ^{13}C n.m.r. (D_2O) δ_C 31.3_7, 31.8_3, 41.0_8, 61.0_1, 61.2_2, 71.3_6, 72.8_0, 73.0_3, 73.8_9, 74.0_7, 82.0_8, 84.0_4, 102.8_1, 175.6_3, 178.5_0.

EXAMPLE 71

6^A-Amino-6^A-deoxy-6^A-N-(3-(N-benzyl)carbamoylpropanoyl) -β-cyclodextrin (β-CDNScNBz)

A solution of β-CDNScNP (Example 65; 140 mg) and benzylamine (17 mg) in DMF (1 ml) was stirred at room temperature for 5 hours. Evaporation *in vacuo* to an oil followed by the addition of acetone (20 ml) with stirring gave a white solid which was collected by vacuum filtration and washed with acetone (2 x 10 ml) and ether (2 x 5 ml). This solid was recrystallized from hot water to give a white solid on cooling. Drying to constant weight over P_2O_5 *in vacuo* gave β-CDNScNBz (125 mg). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CDNScNBz, 1.1. HPLC using a 75% CH_3CN-H_2O eluant showed: t_R (relative to β-CD), β-CDNScNBz, 0.86.

υ_{max} 1653, 1558 cm^{-1}. FAB MS $M+H^+$ requires 1323 found 1323; $M+Na^+$ requires 1345 found 1345. ^{13}C n.m.r. (D_6DMSO) δ_C 34.8_4, 34.9_6, 46.2_2, 64.0_6, 74.1_7, 76.2_1, 76.4_5, 77.0_9, 85.6_6, 85.8_0, 87.7_6, 106.1_0, 106.3_6, 130.9_1, 131.2_9, 132.4_6, 143.5_3, 175.8_8.

EXAMPLE 72

6^A-Amino-6^A-N-(2-N,2-N-(di-2-aminoethyl)-2-aminoethyl)-6^A-deoxy-α-cyclodextrin (α-CDTren)

To a solution of N,N-bis-(2-aminoethyl)-1,2-ethanediamine (40.5 mg) in dry DMF (3 ml) was slowly added a solution of 6^A-

deoxy-6^A-iodo-αu-cyclodextrin (300 mg) in dimethylformamide (6 ml) and the resultant solution was stirred at 70° overnight. The mixture was dried *in vacuo*, the residue was dissolved in water (2 ml) and acetone (30 ml) added. The precipitate was collected by vacuum filtration, rinsed with acetone and dried to give 180 mg of crude product. T.l.c. (solvent B) showed R_C (relative to α-CD) 0.27, 0.22, 0.08. HPLC of the product using a 70% acetonitrile - water eluant showed: t_R (relative to α-CD), 0.85, 1.0, 1.5.

FAB MS M+H$^+$ requires 1102 found 1000, 1101, 1129, 1157. ^{13}C n.m.r (D_2O) 31.0_5, 36.9_3, 38.8_0, 46.3_8, 47.5_4, 50.8_4, 53.6_9, 54.7_3, 55.0_5, 61.6_6, 67.7_9, 72.0_7, 72.9_1, 73.0_9, 74.4_1, 82.4_7, 85.0_1, 102.6_6, 165.4_4.

EXAMPLE 73

6^A-Amino-6^A-N-(3-(N-(2-N,2-N-(di-2-aminoethyl)-2-aminoethyl))-carbamoylpropanoyl)-6^A-deoxy-β-cyclodextrin (β-CDNScTren)

To a solution of β-CDNScNP (Example 65, 300 mg, 0.221 mmol) in dimethylformamide (3 ml) was added N,N-bis(2-aminoethyl)-1,2-ethanediamine (50 mg, 0.34 mmol) in the same solvent. This resulted in the immediate formation of a yellow oil. The mixture was stirred for 2 hours and the solvent was then removed *in vacuo* to give a glassy oil. Trituration of this with acetone (3 x 50 ml) gave a semi-solid which was dissolved in the minimum amount of water (ca. 2 ml). The addition of acetone (50 ml) with stirring gave an off-white precipitate which was collected by vacuum filtration, washed with acetone (2 x 50 ml) and air dried. This procedure was repeated twice. The resulting solid was dissolved in water (50 ml) and stirred with BioRex 70 (3 g, H$^+$ form) for 18 hours. The resin was filtered off and washed with water (2 x 50 ml). The filtrates were discarded and the resin was then rinsed with concentrated ammonia solution (2 x 50 ml). The combined ammonia filtrates were evaporated to about 1 ml and acetone (50 ml) was added with stirring to give a white solid. This was collected by vacuum filtration, washed with acetone and

dried to constant weight *in vacuo* over P_2O_5 to give β-CDNScTren (250 mg, 83%). T.l.c. (solvent B) showed: R_C (relative to β-CD), β-CDNScTren, 0.3. HPLC using a 60% CH_3CNH_2O eluant showed: t_R (relative to β-CD), β-CDNScTren, 2.7.

υ_{max} (KBr disk) 1653 cm^{-1}. FAB MS $M+H^+$ requires 1361 found 1361; $M+Na^+$ requires 1383 found 1383. ^1H n.m.r. (d_6DMSO) 1.8 - 2.8, 3.0 -3.8, 4.9 (s), 5.5 -5.7. ^{13}C n.m.r. 38.0_4, 38.6_7, 39.2_6, 41.1_5, 54.2_1, 56.0_2, 61.3_0, 67.7_9, 73.0_2, 74.2_5, 82.2_0, 84.2_7, $103\ 0_3$, 175.5_9.

EXAMPLE 74

N-((S)-4'-amino-4'-carboxy-butan-1-oyl)-6^A-amino-6^A-deoxy-β-cyclodextrin (βCDGLN)

Step 1: A solution of dicyclohexylcarbodiimide (185.9 mg) in dichloromethane (2 mL) was added in one portion to a stirred solution of N-tBOC-L-glutamic acid-α-^{t}butyl ester (538 mg) in dichloromethane (20 mL). The mixture was left to stir at room temperature for 45 minutes, after which time the mixture was filtered to remove dicyclohexylurea and the clear filtrate was then dried *in vacuo* to give a yellowish oil. This oil was dissolved in dimethylformamide (10 mL) and added to a stirred solution of βCDNH2 (1.057 g) in dimethylformamide (20 mL). The mixture was left to stir at room temperature for 18 hours. The solvent was removed *in vacuo* and the residue triturated with acetone (80 mL) to give an oily solid. This was again evaporated to dryness and the residue triturated with acetone (100 mL). The solid which formed was collected by vacuum filtration, rinsed with acetone (10 mL) and ether (10 mL) and air dried to give 1.34 g of a white solid.

T.L.C (solvent B) showed R_C (relative to βCD) 1.57 plus a faint spot corresponding to βCDNH2

HPLC of the product using 70% acetonitrile-water as eluant showed one peak t_R(relative to βCD) 0.49. FABMS $M+H^+$ requires 1420 found 1420.

Step 2: The product from above (447 mg) was dissolved in anhydrous trifluoroacetic acid (10 mL) and the solution was left to stand at room temperature for 18 hours. The solution was evaporated to dryness and acetone (30 mL) was added to the oily residue. A solution formed and this was evaporated to dryness. Water (10 mL) was added to the residue but none of the residue dissolved. On evaporation of the water, ethanol was added to obtain a white solid. The ethanol was evaporated and the residue was dissolved in water (10 mL). This solution was filtered (0.22 μm) and dropped into acetone (150 mL). The precipitate was collected by vacuum filtration, rinsed with acetone (10 mL) and ether (10 mL) and dried to give 448.7 mg of white powder (81% yield based on starting glutamic acid).

TLC (solvent B) showed R_C(relative to βCD) 0.70. HPLC using 60% acetonitrile-water as eluant showed t_R(relative to βCD) 2.42. FABMS M^+ requires 1263 found 1263.

ν_{max} (KBr disc) 1678, 1645 cm^{-1}. ^{13}Cnmr(D_2O) 175.2_0, 173.0_6, 102.8_6, 84.1_4, 74.0_9, 73.8_5, 73.0_2, 72.8_6, 71.2_9, 67.7_6, 61.2_6, 53.8_1, 41.1_2, 32.1_6, 26.7_9. ^{1}Hnmr (d_6DMSO) 6.1-5.7, 5.1-4.9, 4.7-4,4, 4.0-3.0, 2.4 (t), 2.0 (m).

The procedures, equipment, instruments and chemicals used to measure the chemical hydrolysis of cyclodextrin derivatives in aqueous solution in Examples 75 to 88 were as follows.

Reagents used were of reagent grade unless otherwise stated. Buffer systems (0.2 M carbonate buffers , pH 9.5, 10.0, 10.5, 11.0 and 11.5) were prepared by mixing calculated amounts of $NaHCO_3$ and Na_2CO_3 in H_2O. NaOH or HCl (0.1 M) was used in pH adjustments of buffers with the aid of a Ross pH electrode (Model 81-03, Orion Research) and a pH meter (pHM64a, Radiometer A/S, Copenhagen). The ionic strength of all buffer solutions was adjusted to 0.6 with potassium chloride. The thermoregulator used consisted of a model 1419 Thermomix (B. Braun, W. Germany) and a Tecam incubator. Where a UV detector is mentioned a Waters

model 441 UV absorbance detector at 254 nm was used.

The example below demonstrates the calculations required and preparation of 100 ml of a 0.2 M carbonate buffer, pH 10. Other carbonate buffers required may be made using similar calculations and procedures.

EXAMPLE 75

Preparation of 100 ml of 0.2 M Carbonate Buffer, pH 10.0

pK_2 (H_2CO_3) = 10.32

pH = pK_2 (H_2CO_3) + log ($[CO_3^{2-}]/[HCO_3^-]$)

10.0 = 10.32 + log ($[CO_3^{2-}]/[HCO_3^-]$)

$[CO_3^{2-}]/[HCO_3^-]$ = 0.479

$[CO_3^{2-}]$ = 0.479 $[HCO_3^-]$ (1)

$[CO_3^{2-}] + [HCO_3^-]$ = 0.2 M (2)

1.479 $[HCO_3^-]$ = 0.2 M

$[HCO_3^-]$ = 0.135 M

$[CO_3^{2-}]$ = 0.2 - 0.135 = 0.065 M

FW ($NaHCO_3$) = 84

FW (Na_2CO_3) = 106

0.1 l x 0.135 $mol.l^{-1}$ x 84 $g.mol^{-1}$ = 1.134 g $NaHCO_3$

0.1 l x 0.065 $mol.l^{-1}$ x 106 $g.mol^{-1}$ = 0.689 g Na_2CO_3

Sodium bicarbonate (1.134 g) and sodium carbonate (0.689 g) were added to a 100 ml volumetric flask and water added to make 100 ml.

The measurement of rates of hydrolysis of cyclodextrin derivatives is shown generally below. This example is representative of the procedures used in Examples 76 to 84.

EXAMPLE 76

Measurement of rates of hydrolysis of cyclodextrin derivatives

In a 2 ml volumetric flask the cyclodextrin derivative (5 mg) was dissolved, with or without heating, in water (0.6 ml). Acetonitrile (0.4 ml) was added and the solution equilibrated to

37°C in a water bath. Carbonate buffer (0.2 M, 1 ml), which had been preequilibrated to 37°C, was added and the total mixture was incubated without agitation in a waterbath at 37 ± 0.5°C. At appropriate time intervals, aliquots (50 µl) were withdrawn with a syringe and immediately injected for HPLC assay. The mobile phase was a 70:30 ($^{v}/_{v}$) mixture of redistilled acetonitrile and Milli-Q® water at a flow rate of 1.5 ml.min^{-1}. The disappearance of the cyclodextrin derivative and/or the formation of α- or β-cyclodextrin were followed and the peaks integrated until hydrolysis of the derivative had reached 75%. Hydrolysis rate constants were then calculated using first-order kinetics.

EXAMPLE 77

Hydrolysis of 6^A-O-acetyl-α-cyclodextrin (α-CDOAc)

The rate constants and half-lives for the hydrolysis of α-CDOAc at 37°C are summarized in Table 1. In 0.1 M carbonate buffers in the range of pH 9.5 to 11.0, the following equation with a coefficient of determination (r^2) of 0.9959 was obtained for the pH - rate constant (k) profile of α-CDOAc.

$$\log k = 0.839(pH) - 9.089$$

For monoesterified derivatives of cyclodextrins investigated so far, α-CDOAc and β-CDOAc showed close similarity with each other in hydrolytic stability, whereas both compounds were 5 - 7 times less stable at a given alkaline pH than the prodrugs, including α-CDOIb, β-CDONp and α-CDONp.

Table 1. Rate Constants (k) and Half-lives ($t_{1/2}$) of α-CDOAc in 0.1 M Carbonate Buffer at Different pHs at 37°C.

pH	k, (hr^{-1})	$t_{1/2}$, (hr)
9.5	7.41 x 10-2	9.35
10.0	1.93 x 10-1	3.59
10.5	5.86 x 10-1	1.18
11.0	1.28	0.54

EXAMPLE 78

Hydrolysis of 6^A-O-acetyl-β-cyclodextrin (β-CDOAc)

The rate constants and half-lives for hydrolysis of β-CDOAc at 37°C are summarised in Table 2. In the alkaline pH range of 9.5 to 11.0, the following equation with a coefficient of determination (r^2) of 0.9996 was obtained for the pH rate constant (k) profile:

$$\log k = 0.871(pH) - 9.408$$

Table 2. Rate Constants (k) and Half-lives ($t_{1/2}$) of β-CDOAc in 0.1 M Carbonate Buffers and 0.1 M HCl at 37°C.

Medium	pH	k, hr^{-1}	$t_{1/2}$, hr
Carbonate Buffer	9.5	7.21×10^{-2}	9.61
	10.0	2.04×10^{-1}	3.40
	10.5	5.59×10^{-1}	1.24
	11.0	1.46	0.48
0.1 M HCl	~1.1	4.13×10^{-2}	16.78

EXAMPLE 79

Hydrolysis of 6^A-O-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-α-cyclodextrin (α-CDOIb)

Table 3 gives the rate constants and half-lives for the hydrolysis of α-CDOIb at 3 alkaline pHs at 37°C. In comparison α-CDOIb was found to be approximately 6-8 times more resistant to hydrolysis at a given alkaline pH than β-CDOAc. In an attempt to determine the rate constant for the hydrolysis of this prodrug at gastric pH, the hydrolysis in 0.1 M HCl was followed for one week. Unfortunately , the hydrolysis rate observed was so slow that more and more interfering substances, presumably caused by the competing hydrolysis of the glucosidic linkage of the cyclodextrin moiety, accumulated in the mixture causing difficulties in the quantitization of released α-cyclodextrin. Both of the diastereomeric esters hydrolysed at the same rate. The α-CDOIb peak showed a large overlap with the buffer peak, and was therefore not applicable for kinetic measurement of the hydrolysis.

Table 3. Rate Constants (k) and Half-lives ($t_{1/2}$) of α-CDOIb in 0.1 M Carbonate Buffers at different pHs and 37°C.

pH	k, hr^{-1}	$t_{1/2}$, hr
10.5	8.60×10^{-2}	1.24
11.0	1.73×10^{-1}	0.48
11.5	4.28×10^{-1}	1.62

EXAMPLE 80

Hydrolysis of 6^A-O-((S)-6-methoxy-α-methyl-2-napthaleneacetyl)-α-cyclodextrin (α-CDONp)

In contrast to the poor solubility of β-CDONp, α-CDONp was readily soluble in cool water/acetonitrile (6:4) or water. However, the hydrolysis properties of α-CDONp (Table 4) are still very similar to those of βCDONp in 0.1 M carbonate buffer at three pH's and 37°C.

Table 4. Rate Constants (k) and Half-lives ($t_{1/2}$) of α-CDONp in 0.1 M Carbonate Buffer at Different pHs at 37°C.

pH	k, hr^{-1}	$t_{1/2}$, hr
10.5	1.12×10^{-1}	6.22
11.0	2.33×10^{-1}	2.97
11.5	4.78×10^{-1}	1.45

EXAMPLE 81

Hydrolysis of 6^A-O-((S)-6-methoxy-α-methyl-2-napthaleneacetyl)-β-cyclodextrin (β-CDONp)

Due to the poor solubility of β-CDONp in cold water, heating was necessary for dissolution of this compound. Table 5 shows the rate constants and half-lives for hydrolysis of this compound at pH 11.0 and 11.5 at 37°C. The stability of this prodrug to hydrolysis was not much different from that of α-CDOIb, but was much greater than that of β-CDOAc.

Table 5. Rate Constants (k) and Half-lives ($t_{1/2}$) of β-CDONp in 0.1 M Carbonate Buffer at Different pHs at 37°C.

pH	k, hr^{-1}	$t_{1/2}$, hr
10.5	1.08×10^{-1}	6.40
11.0	2.27×10^{-1}	3.05
11.5	4.45×10^{-1}	1.56

EXAMPLE 82

Difference in Hydrolysis Rate of the Two Diastereoisomers of 6^A-O-(α-methyl-4-(2-methylpropyl)-benzeneacetyl)-β-cyclodextrin (β-CDOIb)

The alkaline hydrolysis of the two diastereoisomers of β-CDOIb, 6^A-O-((S)-α-methyl-4-(2-methylpropyl)-benzeneacetyl)-β-cyclodextrin (β-CDOIb$^+$) and 6^A-O-((R)-α-methyl-4-(2-

methylpropyl)-benzeneacetyl)-β-cyclodextrin (β-CDOIb⁻), was followed by HPLC using an 80% aqueous acetonitrile mobile phase in order to resolve the isomers. The retention times for β-CDOIb⁺, β-CDOIb⁻ and β-CD were 13, 15 and 40 minutes respectively.

In carbonate buffers with pH>10, a significant difference in hydrolysis rates of the two isomers was observed. For example, incubation of 1:1 mixture of β-CDOIb⁺ and β-CDOIb⁻ in 0.1 M sodium carbonate (pH ~ 11.6) at 37°C for 2 hours showed almost complete hydrolysis of β-CDOIb⁻ with concomitant release of β-CD, but only slight hydrolysis of β-CDOIb⁺. After 20 hours of incubation some β-CDOIb⁺ (~10% of total β-CDOIb) remained unhydrolyzed.

The half-life of β-CDOIb⁻ under these conditions is estimated as less than 0.5 hours, and the half-life of β-CDOIb⁺ is 15 to 20 times greater than that of β-CDOIb⁻.

EXAMPLE 83

Hydrolysis of β-CDOIb in 0.1 M Carbonate Buffer (pH 11.0) at 37°C

The hydrolysis of the two diastereomers of β-CDOIb was followed for 18.5 hours by HPLC. The retention times for diastereomer β-CDOIb⁺, β-CDOIb⁻ and β-CD were 8.4, 9.9 and 18.8 minutes. Baseline resolution of β-CDOIb⁺ and β-CDOIb⁻ was unsatisfactory and peak area data was difficult to obtain. Peak height, as an approximation of peak area is also presented as a comparison.

β-CDOIb⁻ was completely hydrolysed after 4 hours, allowing 8 data points to be collected. Table 6 shows the first-order rate constant (k_{1st}), half-life ($t_{1/2}$) and coefficient of determination (r^2) obtained from linear regression based on peak area or peak height.

Table 6. Rate Constants (k), Half-lives ($t_{1/2}$) and Coeffficient of Determination (r^2) of β-CDOIb⁻ in 0.1 M Carbonate Buffers at pH 11.0 and 37°C.

Parameter Used	k_{1st}, (hr^{-1})	$t_{1/2}$, (hr)	r^2
Peak Area	7.01 x 10-1	0.989	0.9748
Peak Height	6.17 x 10-1	1.124	0.9892

The half-life of β-CDOIb⁺ under these conditions is greater than 10 hours.

EXAMPLE 84

Hydrolysis of β-CDOIb in 0.1 M Carbonate Buffer (pH 11.5) at 37°C

The hydrolysis of the two diastereomers of β-CDOIb was followed by HPLC for 24.5 hours with a 73:27 v/v acetonitrile - water mobile phase. The use of UV detection at 254 nm minimised the interference from the carbonate buffer.

β-CDOIb⁻ was completely hydrolysed after 3 hours allowing 7 data points to be collected. After this time a further 9 data points were collected reflecting the hydrolysis rate of β-CDOIb⁺. This hydrolysis rate follows first-order kinetics and linear regression gave the equation below with a coefficient of determination (r^2) of 0.9968:

ln(fraction of β-CDOIb⁺ remaining) = -0.1069 x time (hr) + 0.0282

This equation allowed the calculation of the peak area of β-CDOIb⁺ at times less than 3 hours. The peak area of β-CDOIb⁻ was calculated by subtracting the calculated peak area of β-CDOIb⁺ from the measured total peak area of both diastereomers. The hydrolysis rate of β-CDOIb⁻ follows first-order kinetics and linear regression gave the equation below with a coefficient of determination (r^2) of 0.9978:

ln (fraction of β-CDOIb⁻ remaining) = -1.1369 x time (hr) - 0.0582

Table 7. Rate constant (k_{1st}), the half-life ($t_{1/2}$) and the coefficient of determination (r^2) for the hydrolysis of β-CDOIb⁺ and β-CDOIb⁻ at pH 11.5 and 37°C.

Diastereomer of β-CDOIb	k_{1st}, (hr^{-1})	$t_{1/2}$, (hr)	r^2
β-CDOIb⁺	0.1069	6.483	0.9968
β-CDOIb⁻	1.1369	0.610	0.9978

EXAMPLE 85

Hydrolysis of β-CDOIb in 0.1 N Sodium Hydroxide (pH 13) at Room Temperature

A suspension of β-CDOIb (5.5 mg) in Milli-Q® water (1 ml) was gently heated until all of the solid had dissolved. Sodium hydroxide (0.2 N, 1 ml) was added to the solution. An aliquot of the 0.1 N hydrolysis mixture was filtered (0.45μm) and the hydrolysis followed by HPLC with samples taken every 10 minutes. The hydrolysis of β-CDOIb⁻ was stereoselective and complete in 40 minutes.

EXAMPLE 86

Hydrolysis of β-CDOIb in 0.05 N Hydrochloric Acid (pH 1.3) at 50°C

A suspension of β-CDOIb (5.8 mg) in Milli-Q® water (0.5 ml) was gently heated until all of the solid had dissolved. Hydrochloric acid (0.1 N, 0.5 ml) was added to the solution and the resulting pH 1.3 solution was placed in a water bath maintained at 50°C. The hydrolysis was followed by HPLC. The hydrolysis of β-CDOIb⁻ was stereoselective and complete in 24 hours.

EXAMPLE 87

Isolation of Optically Pure (R)-Ibuprofen by Enantioselective Hydrolysis of β-CDOIb

A diastereomeric mixture of β-CDOIb (1.4 g) was dissolved with heating in water (200 ml) and equilibrated to 37°C. A 0.2 M pH 11.5 carbonate buffer (200 ml), equilibrated to 37°C, was added to the solution with stirring and the mixture incubated at 37°C. The hydrolysis was followed by HPLC using a 73:27 v/v acetonitrile - water mobile phase and a Waters model 441 UV absorbance detector. The retention times for diastereomers A and B were 8.6 and 9.9 minutes respectively. β-CDOIb was selectively hydrolysed to release enriched (R)-Ibuprofen. The reaction was terminated after two hours by adjusting the pH of the solution to 2.0 with 2N hydrochloric acid. The solution was extracted with ether (3 x 200 ml) and the combined organic extracts were dried *in vacuo* to give white crystals. The recovered (R)-Ibuprofen was converted to its corresponding methyl ester and the optical purity measured by ^1H n.m.r. using $Eu(hfc)_3$ (a chiral shift reagent).

Methyl α-methyl-4-(2-methylpropyl)-benzeneacetate ((S,R)-IbOMe, 1.2 mg) enriched in (R)-IbOMe was dissolved in 0.6 ml $CDCl_3$ giving the expected ^1H n.m.r. This solution was poured into a tube containing 3.6 mg $Eu(hfc)_3$. H8 and H9 shifted and H9 split. The H9 splitting shows a predominance of (R)-IbOMe.

0.3 ml of this soln was mixed with 0.3 ml (-)-IbOMe/$Eu(hfc)_3$ in $CDCl_3$. $Eu(hfc)_3$ remains about 2.1 mg/0.6 ml and concentration IbOMe from each solution is the same. A definite splitting occurs with the major isomer (R)-IbOMe being in 3 times excess the minor isomer (S)-IbOMe.

EXAMPLE 88

Hydrolysis of β-CDNScNP in 0.1 M Tris Buffer (pH 7.8) at 37°C

Hydrolysis of the ester was followed by HPLC as previously described, except that a 67:33 v/v mixture of acetonitrile/water

was used as the mobile phase and that a Waters Model 441 UV Absorbance Detector at 254 nm was used for detection of the ester. The retention time of the ester was 5.5 minutes.

The ester showed very rapid hydrolysis at pH 7.8 and 37°C as indicated by the yellow colour of the released 3-nitrophenol. The rate constant (k_{1st}) was calculated to be 5.9542 hr^{-1} which corresponds to a half-life of 7.0 minutes.

The procedures, equipment, instruments, data processing and chemicals used to measure association constants by ultraviolet - visible spectroscopy in Examples 89 to 104 were as follows.

All cyclodextrins were stored in an evacuated dessicator over P_2O_5. All drugs were stored in a dessicator over P_2O_5. All dyes were stored in sealed containers to maintain a known water content determined by microanalysis. All weights were measured on a Mettler AE 160 balance. All spectra were recorded on a Zeiss DMR10 doublebeam spectrophotometer with a thermostatted cell block (± 0.1 K) at 298 K. Cells used for difference spectra experiments were two compartment QS 80 cells, with a pathlength of either 2 x 1.000 cm or 2 x 0.4375 cm. Cells used for straight spectra experiments were single compartment Q1 cells of pathlength 0.201 cm. Between each sample cells were cleaned with acetone, followed by methanol, and sucked dry under gentle tap vacuum. All spectral data was recorded digitally on a microprocessor (SDK80), and analyzed using FORTRAN programs on a SUN 3/60 workstation. All buffers were made using ultra-pure Milli-Q® water as obtained from the Milli-Q® Reagent Water System. Solutions needed to be spectrophotometrically clear, i.e., all solids needed to be completely dissolved without remaining opalescence. If the solutions were not clear they caused scattering of the spectrometer light beam, which caused greater error in each absorbance reading.

Impurities in some of the commercially obtained cyclodextrins could be detected in the UV spectra in the region

300-230 nm. The concentration of this impurity varied with each cyclodextrin. The significant absorbance of these impurities meant that the contribution of the cyclodextrin alone to the overall absorbance in the difference spectra could not be ignored, which made it necessary to measure the extinction coefficients of the cyclodextrin. The inconsistency in the levels of impurities meant that the extinction coefficient needed to be re-measured for each cyclodextrin used.

To calculate an association constant for the formation of a drug-cyclodextrin complex or a dye-cyclodextrin complex the variation of the UV spectrum of the drug or dye in the presence of cyclodextrin should be measured. Because cyclodextrins are U.V. inactive any variation observed in the spectrum would be due to the formation of a complex. Usually this would mean measuring the UV spectra of a series of solutions, with varying cyclodextrin concentration and constant drug concentration, relative to a solvent reference. This straight spectra method was used for some methyl orange systems. In the case of the drugs Naproxen, Ibuprofen, Panadol and Piroxicam, the change in the UV spectrum of the drug due to the addition of cyclodextrin was not sufficient to allow accurate measurement. This was thought to be due to the similarities of the spectra of the drug and the drug-cyclodextrin complex, a view confirmed by the fitted values obtained for the extinction coefficients of the drug-cyclodextrin complex. Thus the difference spectra method was used for these systems, in which the spectrum of the cyclodextrin/drug solution was not measured relative to the solvent, but to a cell with separate compartments containing separated cyclodextrin and drug solutions. By varying the cyclodextrin concentration simultaneously in the sample and reference cells such that the concentrations of drug and cyclodextrin are approximately the same in both cells, the resultant spectrum must be due to the absorbance of the drug-cyclodextrin complex from the mixed drug-cyclodextrin solution. This spectrum may have a magnitude large enough to measure accurately, and if so, a series of spectra could be obtained

which could be used to calcluate an association constant. These difference spectra showed an increase in absorbance as the concentration of cyclodextrin increased. The difference spectra method was also used for the dyes crystal violet and methyl orange.

Buffers were used as the solvent for all experiments. For experiments at pH 6.9, a phosphate buffer of ionic strength 0.1 M was used. This was made by taking 4.4820 g of AR grade $Na_2HPO_4.12H_2O$ and 1.7060 g of A.R. grade KH_2PO_4 and making it up using Milli-Q® water to 500 ml in a volumetric flask. The density of phosphate buffer was determined to be 1.0029 $g.cm^{-3}$ at 298 K (Yin). For experiments at pH 8.6 a tris buffer of ionic strength 0.2 M was used. This was made by taking 55.4 ml of 1.805 M HCl and 44.6859 g of A.R. grade trizma base and making it up using Milli-Q® water to 500 ml in a volumetric flask. For experiments at pH 6.0 a phosphate buffer of ionic strength 0.1 M was used. This was made by taking 1.746 g of AR grade $Na_2HPO_4.12H_2O$ and 4.831 g of A.R. grade KH_2PO_4 and making it up using Milli-Q® water to 500 ml in a volumetric flask. For experiments at pH 7.8 a phosphate buffer was used. This was made by dissolving 6.055 g of Tris-(hydroxy methyl)-aminomethane and 10.55 g of calcium chloride dihydrate in 250 ml of Milli-Q® water, adjusting the pH to 7.8 with HCl, and diluting to 1 litre using Milli-Q® and dry methanol. All stock cyclodextrin and drug solutions were made up by weight, using the density of the buffer to calculate the concentrations. All UV solutions were made by diluting the stock solutions by weight to the appropriate concentration. All solutions in a series were made up and their UV spectra recorded on the same day, for greater experimental consistency. At the start of each experiment a spectral baseline was run. For difference spectra this was a spectrum of drug/solvent versus drug/solvent; for straight spectra this was a spectrum of solvent versus solvent. All spectra were recorded in duplicate, and the absorbance data at the sampled wavelength intervals stored on computer. The two repetitions for each spectrum were averaged, and the averaged baseline then

subtracted.

To calculate the association constant from the spectra data it was necessary to know the extinction coefficients for the cyclodextrin and drug or dye alone, so that the contributions of these species to the total absorbance could be considered. These extinction coefficient values were obtained by recording the spectra of several solutions, say five, of varying concentrations of the drug, dye or cyclodextrin. Each repetition of the sampled spectrum was stored on computer, averaged and a solvent versus solvent baseline subtracted. This data was then linearly regressed to calculate the extinction coefficients at each wavelength. As would be expected, the accuracy of these extinction coefficient values could have a large effect on the fitted values for the extinction coefficients of the cyclodextrin complex, but was not found to affect the fitted value of the association constant greatly.

For most of the systems studied, a system in which one cyclodextrin was complexed with one drug or dye molecule was found. Data from all solutions at one wavelength were fitted separately, to give association constants at each wavelength. Note that it was not possible to calculate association constants at all wavelengths, as it depended on the shape of the spectrum as to whether or not a reasonable fit could be obtained at a particular wavelength. An estimate of the association constant allowed initial calculation of the estimated equilibrium concentrations of drug or dye, cyclodextrin and complex. An estimate of the extinction coefficient of the complex allowed calculation of an estimated absorbance of the sample/reference solution. These values, for each cyclodextrin concentration in the series, could then be compared to the actual experimental values, and new values estimated for the association constant and the extinction coefficient. Via continued iterations until convergence within the specified limit was found, best-fit values could be found for the association constant and the extinction coefficient of the complex. This fitting used a nonlinear least

squares fitting program. Best-fit values for the association constant and the extinction coefficient were found at all suitable wavelengths in the difference spectra.

Calculations used to fit a simple 1:1 model of complex formation:

The equilibrium and conservation equations:

$A + B <-> AB$

$K = [AB] / [A][B]$

$[A] = [A]_0 - [AB]$

$[B] = [B]_0 - [AB]$

where $[A]0$ and $[B]_0$ are the initial concentrations of A and B

[A] and [B] are the equilibrium concentrations of A and B

[AB] is the equilibrium concentra-tion of the complex AB

K is the association constant.

[A], [B] were expressed in terms of [AB], substituted in the expression for the equilibrium constant, and the resulting quadratic equation solved for [AB], the concentration of drug-cyclodextrin complex at equilibrium.

$a = K$

$b = -K([A]_0 + [B]_0 + (1/K))$

$c = K[A]_0[B]_0$

$\Rightarrow [AB] = (-b + (b^2 - 4ac)^{1/2}) / 2a$

The equations describing the absorbance :

Abs(sample cell) = 1(E(A)[A] + E(B)[B] + E(AB)[AB])

Abs(reference cell) = E(A)[A] + E(B)[B] for difference spectra method

Abs(reference cell) = 0 for straight spectra method

Abs(measured) = Abs(sample) - Abs(reference)

where 1 = path length of the cell,

E(A), E(B) = extinction coefficients of A and B,

E(AB) = extinction coefficient of the complex AB.

K/E(AB) are estimated and iterated until convergence is

found.

Theoretically, the association constant should be constant within errors over all wavelengths. The actual values for the association constant were found to vary with wavelength, and thus needed to be averaged to give a final association constant value. From the difference spectrum, regions were chosen which had a reasonable change in absorbance across the cyclodextrin concentration range. The association constant values at these wavelengths were accepted as reasonable to include in the average, and were weighted according to the reciprocal of their error, and a mean value calculated. The root-mean-square deviation of the accepted values could then be calculated to give a final value, with error, for the association constant.

EXAMPLE 89

Measurement of association constant between α-CD and Naproxen

A stock solution of 8.27×10^{-4} M Naproxen and a stock solution of 1.34×10^{-1} M α-CD were made up in phosphate buffer at pH 6.9. A total of 17 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [α-CD] = 6.43, 6.22, 5.92, …, 0.581, 0.311 $\times 10^{-2}$ M
reference [α-CD] = 12.8, 12.4, 11.9, …, 1.16, 0.623 $\times 10^{-2}$ M
sample [Naproxen] = $(4.135 \pm 0.005) \times 10^{-4}$ M
reference [Naproxen] = 8.27×10^{-4} M

The spectrum of each sample/reference solution pair was recorded in 2.000 cm pathlength cells over 340-280 nm, sampling at 1.0 nm intervals, using an integration time of 6.4 seconds per wavelength and a band width of 1.0 nm. Fitted values in the region 340-333 nm and 294-288 nm were averaged to give an association constant of 16 ± 1.

EXAMPLE 90

Measurement of association constant between β-CD and Naproxen

A stock solution of 8.92×10^{-4} M Naproxen and a stock solution of 1.58×10^{-2} M β-CD were made up in phosphate buffer at pH 6.9. A total of 16 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD]=7.71, 7.04, 6.44, … , 0.643, 0.331 $\times 10^{-3}$ M
reference [β-CD]=15.4, 14.1, 12.9, … , 1.29, 0.662 $\times 10^{-3}$ M
sample [Naproxen] = $(4.455 \pm 0.005) \times 10^{-4}$ M
reference [Naproxen]=8.92×10^{-4} M

The spectrum of each sample/reference solution pair was recorded in 2.000 cm pathlength cells over 340-280 nm, sampling at 1.0 nm intervals, using an integration time of 6.4 seconds per wavelength and a band width of 1.0 nm. Fitted values in the region 339-334 nm and 293-289 nm were averaged to give an association constant of 670 ± 40.

EXAMPLE 91

Measurement of association constant between γ-CD and Naproxen

A stock solution of 7.99×10^{-4} M Naproxen and a stock solution of 1.2×10^{-2} M γ-CD were made up in phosphate buffer at pH 6.9. A total of 23 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [γ-CD]=5.75, 5.50, 5.17, … , 0.487, 0.242 $\times 10^{-3}$ M
reference [γ-CD]=11.4, 10.9, 10.3, … , 0.970, 0.484 $\times 10^{-3}$ M
sample [Naproxen] = $(3.95 \pm 0.05) \times 10^{-4}$ M
reference [Naproxen] = 7.99×10^{-4} M

The spectrum of each sample/reference solution pair was recorded in 2.000 cm pathlength cells over 350-280 nm, sampling at 1.0 nm intervals, using an integration time of 6.4 seconds per wavelength and a band width of 1.0 nm. Fitted values in the

region 340-334 nm and 293-288 nm were averaged to give an association constant of 120 ± 10.

EXAMPLE 92

Measurement of association constant between dimethyl-β-cyclodextrin (DIMEB) and Naproxen

A stock solution of 7.68×10^{-4} M Naproxen and two stock solutions of 1.44×10^{-1} M and 2.88×10^{-2} M DIMEB were made up in phosphate buffer at pH 6.9. A total of 20 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [DIMEB]=6.69, 5.82, 4.80, … , 0.219, 0.102 $\times 10^{-2}$ M
reference [DIMEB]=13.4, 11.6, 9.61,…, 0.439, 0.204 $\times 10^{-2}$ M
sample [Naproxen]=3.84 $\times 10^{-4}$ M
reference [Naproxen]=7.68 $\times 10^{-4}$ M

The spectrum of each sample/reference solution pair was recorded in 0.8750 cm pathlength cells over 340-280 nm, sampling at 1.0 nm intervals, using an integration time of 1.6 seconds per wavelength and a slit width of 0.4 nm. Fitted values in the region 340-331 nm, 325-321 nm and 289-285 nm were averaged to give an association constant of 510 ± 80.

EXAMPLE 93

Measurement of association constant between β-$CDNH_2$ and Naproxen

A stock solution of 7.68×10^{-4} M Naproxen and a stock solution of 2.03×10^{-2} M β-$CDNH_2$ were made up in phosphate buffer at pH 6.9. A total of 14 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-$CDNH_2$]=9.45, 8.50, 7.51, … , 2.15, 1.66 $\times 10^{-3}$ M
reference [β-$CDNH_2$]=18.9, 17.0, 15.0, …, 4.29, 3.32 $\times 10^{-3}$ M
sample [Naproxen]=3.84 $\times 10^{-4}$ M
reference [Naproxen]=7.68 $\times 10^{-4}$ M

The spectrum of each sample/reference solution pair was recorded in 0.8750 cm pathlength cells over 340-280 nm, sampling at 1.0 nm intervals, using an integration time of 1.6 seconds per wavelength and a slit width of 0.6 nm. Fitted values in the region 340-331 nm, 319 nm and 291-285 nm were averaged to give an association constant of 640 ± 100.

EXAMPLE 94

Measurement of association constant between β-CDN4N and Naproxen

A stock solution of 7.80×10^{-4} M Naproxen and a stock solution of 4.23×10^{-2} M β-CDN4N were made up in tris buffer at pH 8.6. A total of 15 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CDN4N]=19.6, 17.9, 15.8, … , 3.73, 2.78 $\times 10^{-3}$ M
reference [β-CDN4N]=39.2, 35.8, 31.6, …, 7.47, 5.55 $\times 10^{-3}$ M
sample [Naproxen]=3.90 $\times 10^{-4}$ M
reference [Naproxen]=7.80 $\times 10^{-4}$ M

The spectrum of each sample/reference solution pair was recorded in 0.8750 cm pathlength cells over 340-270 nm, sampling at 1.0 nm intervals, using an integration time of 1.6 seconds per wavelength and a slit width of 1.0 nm. Fitted values in the region 340-332 nm, 323 nm and 290-287 nm were averaged to give an association constant of 150 ± 30.

EXAMPLE 95

Measurement of association constant between β-CD and Ibuprofen

A stock solution of 6.92×10^{-3} M Ibuprofen and a stock solution of 1.49×10^{-2} M β-CD were made up in phosphate buffer at pH 6.9. A total of 18 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD] = 7.44, 6.43, 6.09, … , 1.39, 1.02 $x10^{-3}$ M
reference [β-CD] = 14.9, 12.8, 12.2, … , 2.75, 2.02 $x10^{-3}$ M
sample [Ibuprofen] = (3.455 ± 0.005) $x10^{-3}$ M
reference [Ibuprofen] = 6.92 $x10^{-3}$ M

The spectrum of each sample/reference solution pair was recorded in 2.000 cm pathlength cells over 280-250 nm, sampling at 0.5 nm intervals, using an integration time of 6.4 seconds per wavelength and a band width of 1.0 nm. Fitted values in the region 277-274 nm were averaged to give an association constant of 2900 ± 500.

EXAMPLE 96

Measurement of association constant between DIMEB and Ibuprofen

A stock solution of $7.00x10^{-3}$ M Ibuprofen and three stock solutions of 4.37×10^{-2} M, $8.91x10^{-3}$ M and $4.47x10^{-3}$ M DIMEB were made up in phosphate buffer at pH 6.9. A total of 16 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [DIMEB]=21.8, 10.5, 5.21, …, 0.997, 0.495 $x10^{-3}$ M
reference [DIMEB]=43.7, 20.8, 10.5, …,1.98, 0.992 $x10^{-3}$ M
sample [Ibuprofen]=3.43 - 3.51 $x10^{-3}$ M
reference [Ibuprofen]=7.00 $x10^{-3}$ M

The spectrum of each sample/reference solution pair was recorded in 2.000 cm pathlength cells over 280-268 nm, sampling at 0.5 nm intervals, using an integration time of 3.2 seconds per wavelength and a slit width of 1.0 nm. Fitted values in the region 277-275 nm were averaged to give an association constant of 9100 ± 500.

EXAMPLE 97

Measurement of association constant between DIMEB and Piroxicam

A stock solution of 1.96×10^{-4} M Piroxicam and a stock solution of 1.49×10^{-1} M DIMEB were made up in phosphate buffer. A total of 20 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [DIMEB] = 7.08, 6.83, 6.65, … , 1.33, 0.970 $\times 10^{-2}$ M
reference [DIMEB] = 14.1, 13.7, 13.3, …, 2.67, 1.93 $\times 10^{-2}$ M
sample [Piroxicam] = 9.785 ± 0.005 $\times 10^{-5}$ M
reference [Piroxicam] = 1.96 $\times 10^{-4}$ M

The spectrum of each sample/reference solution pair was recorded in 0.8750 cm pathlength cells over 420-330 nm, sampling at 1.0 nm intervals, using an integration time of 3.2 seconds per wavelength and a slit width of 0.8 nm. Fitted values in the region 420-391 nm were averaged to give an association constant of 53 ± 1. When values in the region 390-386 nm and 349-348 nm were included in the average the association constant was averaged to be 53 ± 14.

EXAMPLE 98

Measurement of association constant between β-CD and Panadol

A stock solution of 3.96×10^{-4} M Panadol and a stock solution of 1.17×10^{-2} M β-CD were made up in phosphate buffer at pH 6.9. A total of 18 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD] = 4.90, 4.61, 4.31, …, 0.215, 0.0998 x 10^{-3} M
reference [β-CD] = 9.78, 9.23, 8.63,…, 0.421, 0.212 x 10^{-3} M
sample [Panadol] = (1.982 ± 0.002) x 10^{-4} M
reference [Panadol] = 3.96 x 10^{-4} M

The spectrum of each sample/reference solution pair was

recorded in 0.8750 cm pathlength cells over 300-220 nm, sampling at 1.0 nm intervals, using an integration time of 3.2 seconds per wavelength and a band width of 1.0 nm. Fitted values in the region 267-250 nm were averaged to give an association constant of 130 ± 10.

EXAMPLE 99

Measurement of association constant between DIMEB and Panadol

A stock solution of 3.94×10^{-4} M Panadol and a stock solution of 1.005×10^{-3} M DIMEB were made up in phosphate buffer at pH 6.9. A total of 19 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [DIMEB] = 5.03, 4.60, 4.30, …, 0.207, 0.100 x 10^{-3} M

reference [DIMEB] = 10.1, 9.20, 8.60,…,0.413, 0.200 x 10^{-3} M

sample [Panadol] = (1.972 ± 0.002) x 10^{-4} M

reference [Panadol] = 3.94 x 10^{-4} M

The spectrum of each sample/reference solution pair was recorded in 0.8750 cm pathlength cells over 300-220 nm, sampling at 1.0 nm intervals, using an integration time of 3.2 seconds per wavelength and a band width of 1.0 nm. Fitted values in the region 259-255 nm were averaged to give an association constant of 83 ± 3. When values in the region 261-260 nm, 254 nm and 232-224 nm were included in the average the association constant was averaged to be 110 ± 50.

EXAMPLE 100

Measurement of association constant between β-cyclodextrin and Crystal Violet

A stock solution of 1.56×10^{-4} M Crystal Violet and a stock solution of 1.60×10^{-2} M β-CD were made up in phosphate buffer at pH 6.9. A total of 16 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD]=11.7, 12.7, 14.2, … , 0.828, 0.778 x10^{-3} M
reference [β-CD] = 0
sample [Crystal Violet] = 1.55 x 10^{-5} M
reference [Crystal Violet] = 3.10 x10^{-5} M

The spectrum of each sample was recorded in 0.8750 cm pathlength cells over 650-550 nm, sampling at 1.0 nm intervals, using an integration time of 1.6 seconds per wavelength and a slit width of 0.8 nm. Fitted values in the region 646-612 nm were averaged to give an association constant of 2400 ± 200.

EXAMPLE 101

Measurement of association constant between β-CDNH$_2$ and Crystal Violet

A stock solution of 1.56x10^{-4} M Crystal Violet and a stock solution of 3.00x10^{-2} M β-CDNH$_2$ were made up in phosphate buffer at pH 6.9. A total of 15 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD]=24.8, 19.7, 14.6, … , 0.925, 0.865 x10^{-3} M
reference [β-CD] = 0
sample [Crystal Violet] = 1.63 x 10^{-5} M
reference [Crystal Violet] = 3.25 x10^{-5} M

The spectrum of each sample was recorded in 0.8750 cm pathlength cells over 650-550 nm, sampling at 1.0 nm intervals, using an integration time of 1.6 seconds per wavelength and a slit width of 0.8 nm. Fitted values in the region 650-617 nm were averaged to give an association constant of 680 ± 50, whereas if values in the region 590-566 nm were included in the average the association constant was averaged to be 800 ± 800. A repeat of this experiment over a different range of β-CDNH$_2$ concentrations gave very similar results.

EXAMPLE 102

Measurement of association constant between β-CD and Methyl Orange

A stock solution of 1.05×10^{-4} M Methyl Orange and a stock solution of 1.49×10^{-2} M β-CD were made up in phosphate buffer at pH 6.0. A total of 16 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD]=7.03, 6.59, 6.02, … , 0.419, 0.0857 $\times 10^{-3}$ M
reference [β-CD] = 0
sample [Methyl Orange] = $(5.235 \pm 0.001) \times 10^{-5}$ M
reference [Methyl Orange] = 10.5×10^{-5} M

The spectrum of each sample was recorded in 0.8750 cm pathlength cells over 550-400 nm, sampling at 1.0 nm intervals, using an integration time of 1.6 seconds per wavelength and a slit width of 0.8 nm. Fitted values in the region 550-468 nm and 435-424 nm were averaged to give an association constant of 3300 ± 600.

EXAMPLE 103

Measurement of association constant between β-CDNH$_2$ and Methyl Orange

A stock solution of 1.05×10^{-4} M Methyl Orange and a stock solution of 2.81×10^{-2} M β-CD were made up in phosphate buffer at pH 6.0. A total of 15 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [β-CD]=12.8, 12.0, 10.9, … , 0.845, 0.788 $\times 10^{-3}$ M
reference [β-CD] = 0
sample [Methyl Orange] = $(5.236 \pm 0.001) \times 10^{-5}$ M
reference [Methyl Orange] = 10.5×10^{-5} M

The spectrum of each sample was recorded in 0.8750 cm pathlength cells over 550-400 nm, sampling at 1.0 nm intervals,

using an integration time of 1.6 seconds per wavelength and a slit width of 0.8 nm. Fitted values for a 1:1 model in the regions 550-460 nm and 440-433 nm were averaged to give an association constant of 500 ± 500. However it was found that a model where the Methyl Orange is complexed by two cyclodextrins could be fitted. Fitted values for a 2:1 model in the region 530-480 nm were averaged to give association constants of K_1 = 1900 ± 300 and K_2 = 23 ± 4.

EXAMPLE 104

Measurement of association constant between α-CD_2NSc and Methyl Orange

A stock solution of 6.09×10^{-4} M Methyl Orange and a stock solutions of 2.99×10^{-3}M and 3.09×10^{-4}M α-CD_2NSc were made up in phosphate buffer at pH 6.0. A total of 20 spectra were run, both sample and reference solutions being made up by weight dilutions of the stocks.

sample [α-CD_2NSc]=19.8, 17.9, 16.0, ..., 0.827, 0.726 $\times 10^{-4}$ M
sample [Methyl Orange] = (2.030 ± 0.001) x 10^{-4} M

The spectrum of each sample was recorded in 0.201 cm pathlength cells over 500-400 nm, sampling at 1.0 nm intervals, using an integration time of 6.4 seconds per wavelength and a slit width of 0.4 nm. Fitted values in the region 480-460 nm and 439-435 nm were averaged to give an association constant of 3600 ± 1600.

The procedures, equipment, instruments, data processing and chemicals used to titrate the cyclodextrin derivatives in Examples 105 to 111 were as follows.

The glass titration vessel, about 100 ml in volume, has three vertical holes for nitrogen purging tube, pH electrode and receiving solution from the burette respectively. It has a water jacket connected to a thermostatted water bath to keep the temperature constant during titration processes. A teflon coated

magnetic stirring bar, ~1.5 cm long, was used to homogenize the solution after each adding of the standard solution (titrant). ROSS pH electrode (model No. 81-03, Orion Research Incorporated) and a pH meter (PHM64a, Radiometer A/S Copenhagen) with precision of 0.001 pH unit were used in all the reported pH measurements.

HCl and/or NaOH solutions are used as titrants in all the measurements of pKa's of cyclodextrin derivatives. They are prepared and standardized in the following way.

HCl solution was standardized as follows. Dilute from any higher concentration of HCl solution to about 0.005 M in a 500 ml volumetric flask using Milli-Q® water. Standardize the solution with Borax (disodium tetraborate, AR) to the precision of 0.001 millimolar. The end point is at about pH 5.1, for which Methyl red is a suitable indicator. However, since the color change is not sharp enough for standardizing such a diluted solution to the required precision, which is commonly <0.1%, a pH electrode was used to determine the end point. Meanwhile, the initial volume of the Borax solution in the vessel has to be controlled with a pipet, and the weight of Borax samples for repetitions have to be within the accuracy of 0.001 g. The Milli-Q® water used to make Borax solution was bubbled with high purity nitrogen gas for one hour.

Carbon dioxide free NaOH was prepared as follows. Weigh about 5 grams AR grade NaOH into a small volumetric flask, add 5 ml Milli-Q® water, stand by for 24 hours. Decant the solution into 1 litre nitrogen bubbled Milli-Q® water. Standardize this carbon dioxide free NaOH solution in the normal way with potassium hydrogen phthalate.

A phosphate buffer solution (0.025 M disodium hydrogen ortho-phosphate and 0.025 M potassium dihydrogen phosphate) of pH ~7 is used to standardize the pH meter since the iso pH of the ROSS electrode is 7. Either 0.05 M potassium hydrogen phthalate solution (pH ~4) or 0.01 M disodium tetraborate solution (pH ~9)

can be used to determine the slope depending on the pH range of the measurement. Generally the pH 4 buffer is preferred because of the interference of carbon dioxide at high pH.

The direct pH titrations for the cyclodextrin derivatives are performed in the normal way. 0.5 ml increments of the titrants produces sufficient number of data points for high precision data fitting. The results of pKa and the fitted purities are usually checked with back titrations, in which acid or base of high concentration is added (normally not more than 10 ml) to reach the pH value of the final end point estimated from a direct titration before the recorded titration is performed in the normal manner. If the sample is a base, nitrogen purging for half an hour follows the acidification before the back titration with the NaOH. The temperature was held at 25.0 °C for all the experiments reported below.

The relationship between the volume of the added titrant and the resulted pH value can be derived from the acid dissociation constant expressions and the charge and mass balance equations. The theoretical titrant volume is expressed as a non-linear function of the corresponding concentration of free acid (converted from the measured pH value), with the total amount of sample used (in millimole) and the initial volume (millilitre) of the solution as the fixed parameters and the estimated acid dissociation constants as the variable parameters. The calculated volumes are compared to the experimental values using a non-linear least squares fitting computer program. The new values are calculated via reestimation of the unknown acid dissociation constants in continuous iteration until convergence (within the specified limit of error) is found between the measured and calculated titrant volumes. This process allows the establishment of the best-fit values for the acid dissociation constants of the chemicals titrated.

Prior to the fitting process, the measured pH values are corrected for electrode error. The slopes and the intercepts of

the linear equation for the pH correction are experimentally determined for different conditions (mainly ionic strength) by titrating standardized HCl with standardized NaOH. The pK_w for different ionic strength are also determined by the same experiments and the appropriate value is used in the fitting.

[potassium chloride] = 0.5, T = 25.0 °C
pH(corrected) = 1.0035 x pH(measured) + 0.085, pKw = 13.90
[potassium chloride]=0.0, T = 25.0 °C
pH(corrected) = 0.9999 x pH(measured) + 0.142, pKw = 14.16

In all the direct titrations of cyclodextrin amine derivatives with HCl, electrode drifting after the pH passes the pKa of a mono amine or the second pKa of a diamine has been a cause of difficulties in titration and significant uncertainties among the repetitions. It took up to 1.5 hours for the electrode to stablize after drifting from lower to higher pH at one data point in the worst case. And very often it ended up with a pH value higher than that of a few steps back. Depending on the allowed stabilizing time in the repetitive titrations of one cyclodextrin amine, the fitted pKa's could vary by ~0.4 pH unit, as happened for β-CDN4N.

EXAMPLE 105

Titration of β-$CDNH_3$.Cl

Pure β-$CDNH_3Cl$ (0.1371 g) and potassium chloride (1.67 g) were dissolved in Milli-Q® water (40.0 ml) in the titration vessel. 0.005765 M sodium hydroxide containing 0.5 M potassium chloride was used to titrate the sample solution from pH 4.5 to 10.1. The fitted pKa is 8.83 ± 0.01 at 25°C and an ionic strength of 0.5.

EXAMPLE 106

Back titration of α-$CDNH_2$

α-$CDNH_2$ (0.1216 g) and potassium chloride (1.6716 g) were

dissolved in nitrogen bubbled Milli-Q® water (40.0 ml). 0.36 ml of 0.36 M HCl solution was added to neutralize the titrant to pH 3.7. After standing for 2 hours 0.005855 N NaOH containing 0.5 M potassium chloride was used to titrate the sample solution to pH 10.435. The initial volume was 40.0 ml + 0.36 ml + 2.20 ml (NaOH used to neutralize the excessive hydrogen ion introduced in the acidification process). The fitted pKa is 8.81 ± 0.01 under the condition of 25.0 °C and 0.5 ionic strength.

EXAMPLE 107

Direct titration of β-CDNSc

β-CDNSc (0.1259 g) and potassium chloride (1.67 g) were dissolved in Milli-Q® water (40.0 ml). 0.005765 M NaOH containing 0.5 M potassium chloride was used to titrate the sample solution from pH 3.6 to pH 10.5. The fitted pKa is 4.69 ± 0.02 under the conditions of 25.0 °C and 0.5 ionic strength.

EXAMPLE 108

Back titration of β-CDNSc

β-CDNSc (0.1481 g) and potassium chloride (1.85 g) were dissolved in Milli-Q® water (40.0 ml). 1.02 ml 0.1171 N NaOH containing 0.5 M potassium chloride was added and pH was 9.97. 0.004945 N HCl with 0.5 M potassium chloride was used to titrate the sample solution from pH 9.97 to 3.4. The initial volume was 40.0 ml + 1.02 ml + 3.52 ml (HCl used to neutralize the excessive hydroxide ion introduced in the adding of NaOH). The fitted pKa is 4.60 ± 0.006 under the conditions of 25.0 °C and 0.5 ionic strength.

The average of the above results gives the pKa for β-CDNSc as 4.65 ± 0.02.

EXAMPLE 109

Direct titration of β-CDNGl

β-CDNGl (0.1310 g) and potassium chloride (1.67 g) were dissolved in Milli-Q® water (40.0 ml) in the titration vessel. 0.005765 M CO_2 free NaOH containing 0.5 M potassium chloride was

used to titrate the sample solution from pH 3.6 to pH 10.29. The fitted pKa is 4.70 ± 0.01.

EXAMPLE 110

Back titration of β-CDN4N

β-CDN4N (0.1192 g) and potassium chloride (1.67 g) were dissolved in 0.004945 M hydrochloric acid (40.0 ml). The molar ratio of β-CDN4N to hydrochloric acid is 1:1 on the basis of 100% purity for β-CDN4N. After standing for 2 hours, 0.005765 M sodium hydroxide containing 0.5 M potassium chloride was used to titrate the sample solution from pH 5.5 to pH 10.1. The fitted pKa_1 is 8.56 ± 0.02, pKa_2 is 10.33 ± 0.01 at 25°C and an ionic strength of 0.5.

EXAMPLE 111

Back titration of β-CDN6N

β-CDN6N (0.0914 g) and potassium (1.67 g) chloride were dissolved in Milli-Q® water (40.0 ml). 0.36 M HCl (0.42 ml) was added to acidify the sample solution to pH 3.5. After standing for at least 2 hours 0.005855 M NaOH containing 0.5 M potassium chloride was used to titrate the solution to pH 10.5. The initial volume was 40.0 ml + 0.42 ml + 2.67 ml (NaOH used to neutralize the excessive hydrogen ion introduced in the acidification). The fitted pKal is 8.87 ± 0.005, pKa2 is 10.15 ± 0.005 under the conditions of 25.0°C and 0.5 ionic strength.

The procedures, equipment, instruments, data processing and chemicals used to measure association constants by fluorescence spectroscopy in Examples 112 to 118 were as follows.

A Perkin-Elmer 3000 Fluorescence Spectrometer is used in all the experiments of fluorescence measurement. The sample cuvette, 1 cm^2, is not thermostatted. In the experiments using TNS as the fluorophore, the excitation wavelength is 366 nm and a cut off filter at 390 nm is installed in the emission light path. The intensities of the fluorescence of all samples are measured at the peak wavelength which should be searched between 400 nm to

500 nm for each different cyclodextin.

TNS potassium salt (SIGMA) was dissolved in a phosphate buffer (0.025 M potassium dihydrogen phosphate + 0.025 M di-sodium hydrogen orthophosphate, pH=6.9, I=0.1) to make a stock solution of 5.007×10^{-5} M. αCD and β-CD were suplied by Nihon Shckuhin Kako Co..

The fluorescence spectrum and the quantum yield of many fluorescing materials are sometimes dependent on the local molecular environment. For example, the fluorescing probe molecule used in the following experiments, 2-p-toluidinyl-naphthalene-6-sulphonate (TNS), is essentially non-fluorescent in water, but is highly fluorescent when dissolved in nonpolar solvents or when bound to macromolecules. It has been used as a microprobe for conformation changes in the proteins to which it binds.

It was also found that TNS can be included in the annuli of some cyclodextrins or cyclodextrin polymers in which cases the fluorescence of TNS in aqueous solution is enhanced to different extend depending upon the including molecules and their concentrations in the solution [A. Harada et.al. Macromolecules 10; 676-681 (1977)]. This observation allows the measurement for the stability constant of the inclusion by varying the concentration of one cyclodextrin while keeping the TNS concentration constant.

Since the fluorescence intensity of the unincluded TNS molecules in the aqueous solution can be neglected, the measured fluorescence intensities at the presence of various cyclodextrins can be approximated to be proportional to the concentration of the included TNS molecules, e.g., [CD-TNS] in the case of 1:1 inclusion, or [CD_2-TNS] in the case of 2:1 inclusion, etc.

Model for 1:1 inclusion:

When the concentration of the free cyclodextrin, $[CD]_f$, is

always much greater than that of complexed cyclodextrin, [CD-TNS], the measured fluorescence intensity, I, has the following relation with the total concentration of cyclodextrin, [CD]:

$$I = I_{inf} K[CD] / (1+K[CD])$$

where K is the stability constant, I_{inf} is fluorescence intensity at infinite concentration of cyclodextrin. The nonlinear fit of the measured I vs [CD] should yield the estimation of K.

The double reciprical form of the above equation, i.e., 1/I as a linear function of I/[CD], is sometimes applied to comfirm the 1:1 ratio of the inclusion. The stability constant can also be calculated from the fitted slope of the straight line.

Model for 2:1 inclusion:

When $[CD]_f$ is always much greater than $[CD_2-TNS]$, I is related to [CD] as the following:

$$I=(\alpha K_1[CD]+K_1K_2[CD]^2) I_{inf} / (1+K_1[CD]+K_1K_2[CD]^2)$$

where K_1 is the first stability constant for the 1:1 inclusion at lower cyclodextrin concentration, and K_2 is the second stability constant for the 2:1 inclusion at higher cyclodextrin concentration. α is the ratio of the molar fluorescence enhancement by the 1:1 complex (CD-TNS) to the molar enhancement by the 2:1 complex (CD_2-TNS). The three unknown parameters, K_1, K_2 and α, are estimated simultaneously when a non-linear fitting is performed with the measured I vs. [CD].

The non-linear fitting subroutine used in all the data analysis for the fluorescence experiments is DATAFIT by Dr T. Kurucsev, interfaced by a main program called FLUO.NLF.F.

EXAMPLE 112

Measurement of fluorescence of α-CD with TNS

The weak fluorescence of the mixture of TNS and α-CD has no spectral shift compared to that of TNS in buffer only. No enhancement in intensity was observed with [CD] in the range from $1.0x10^{-5}$ M to $1.0x10^{-3}$ M ([TNS]=$1.0x10^{-6}$ M in all samples). Conclusion: the stability constant of the inclusion of TNS by α-CD is too small to be measured with this method, or the inclusion has no effect on the fluorescence of TNS.

EXAMPLE 113

Measurement of fluorescence of α-CD_2NSc with TNS

The weak fluorescence of the mixture of TNS and α-CD_2NSc has no spectral shift compared to that of TNS in buffer only. No enhancement in intensity was observed with [CD] in the range from $1.0x10^{-5}$ M to $1.0x10^{-3}$ M ([TNS]=$1.0x10^{-6}$ M in all samples). Conclusion: the stability constant of the inclusion of TNS by α-CD_2NSc is too small to be measured with this method, or the inclusion has no effect on the fluorescence of TNS.

EXAMPLE 114

Measurement of fluorescence of β-CD with TNS

Two β-CD stock solutions of 0.010 M and 0.00010 M respectively, were prepared by (1) disolving 0.2841 g β-CD in 25.0323 g phosphate buffer solution (pH=6.9, I=0.1); and (2) diluting 0.2565 g of the above β-CD solution to 25.6496 g using the same buffer solution. 33 sample solutions, 5 ml of each, were prepared by mixing the appropriate β-CD stock solutions and the buffer with 0.50 ml of $5.007x10^{-5}$ M TNS stock solution so that the [TNS] in all samples were $5.0x10^{-6}$ M, and [CD] were in 2:1, 2.5:1, 3:1, …, 10:1, 12.5:1, …, 100:1, 120:1, …, 500:1, …, 1000:1, and 1200:1 ratio to [TNS]. The peak of the fluorescence was found to be at 455 nm. The fluorescence intensities at this wavelength, increasing with the increase of [CD], were measured for all the sample solutions.

The data fitted with the equation of 2:1 inclusion model

very well, resulting two stability constants K_1 (for 1:1 inclusion) as 4000 ± 286 M^{-1} and K_2 (for 2:1 inclusion) as 84 ± 5 M^{-1} respectively.

EXAMPLE 115

Measurement of fluorescence of β-CD_2NOx with TNS

β-CD_2NOx stock solution of 0.001001 M was prepared by disolving 0.0891 g of β-CD_2NOx in 19.1668 g phospate buffer solution (pH=6.9, I=0.1), then diluting 15.39 ml of the above solution to 30.77 ml with the same buffer solution. 18 sample solutions, 6 ml of each, were prepared by mixing the appropriate amount of β-CD_2NOx stock and the buffer solutions with 0.12 ml of $5.007x10^{-5}$ M TNS stock solution so that the [TNS] in all samples were $1.0x10^{-6}$ M, and [CD] were in 2.75:1, 5:1, 10:1, …, 100:1, …, 300:1 ratio to [TNS]. The peak of the fluorescence was found to be at 425 nm. The fluorescence intensities at 430 nm, increasing with the increase of [CD], were measured for the sample solutions.

The data fitted with equation of 1:1 inclusion model very well, resulting a single stability constant K as 29000 ± 312 M^{-1}.

EXAMPLE 116

Measurement of fluorescence of β-CD_2NSc with TNS

β-CD_2NSc stock solution of 0.00200 M was prepared by dissolving 0.0956 g β-CD_2NSc in 20.3359 g phosphate buffer solution (pH=6.9, I=0.1). 20 sample solutions, 2.0 ml of each, were prepared by mixing the appropriate amount of β-CD_2NSc stock and the buffer solutions with 0.040 ml of $5.007x10^{-5}$ M TNS stock solution so that the [TNS] in all samples were $1.0x10^{-6}$ M, and [CD]s were in 10:1, 15:1, 20:1, …, 100:1, …, 1000:1 ratio to [TNS]. The peak of the fluorescence was found to be at 438 nm. The fluorescence intensities at this wavelength, increasing with the increase of [CD], were measured for all the sample solutions.

The data fitted with the equation of 1:1 inclusion model

very well, resulting a single stability constant K as 15700 ± 258 M^{-1}.

EXAMPLE 117

Measurement of fluorescence of β-CD_2NGlu with TNS

β-CD_2NGlu stock solution of 0.00200 M was prepared by dissolving 0.0978 g β-CD_2NGlu in 20.6832 g phosphate buffer solution (pH=6.9, I=0.1). 22 sample solutions, 5 ml of each, were prepared by mixing the appropriate amount of β-CD_2NGlu stock and the buffer solutions with 0.10 ml of 5.007×10^{-5} M TNS stock solution so that the [TNS] in all samples were 1.00×10^{-6} M, and [CD] were in 10:1, 15:1, 20:1, 100:1, 125:1, …, 1000:1 ratio to [TNS]. The peak of the fluorescence was found to be at 442 nm. The fluorescence intensities at this wavelength, increasing with the increase of [CD], were measured for all the sample solutions.

The data fitted with the equation of 1:1 inclusion model very well, resulting a single stability constant K as 7800 ± 184 M^{-1}.

EXAMPLE 118

Measurement of fluorescence of α,β-CD_2NSc with TNS

α,β-CD_2NSc stock solution of 0.00100 M was prepared by dissolving 0.0876 g α,β-CD_2NSc in 40.0023 g phosphate buffer solution (pH=6.9, I=0.1). 22 sample solutions, 5 ml of each, were prepared by mixing the appropriate amount of α,β-CD_2NSc stock and the buffer solutions with 0.10 ml of 5.005×10^{-5} M TNS stock solution so that the [TNS] in all samples were 1.00×10^{-6} M, and [CD] were in 10:1, 15:1, 20:1, … 100:1, 125:1, … 1000:1 ratio to [TNS]. The peak of the fluorescence was found to be at 445 nm. The fluorescence intensities at this wavelength, increasing with the increase of [CD], were measured for all the sample solutions.

The data fitted with the equation of 1:1 inclusion model very well, resulting a single stability constant K as 1027 ± 5 M^{-1} .

The procedures, equipment, instruments, data processing and

chemicals used to measure association constants of drugs by liquid chromatography in Example 119 is as follows.

HPLC was carried out using an ICI LC1500 pump connected to a Waters Lambda-Max 481 detector. Samples were injected using an ICI LC1600 autosampler controlled by an ICI DP800 Data Station. The column used was a Merck Hibar Lichrosorb Diol column (4 x 250mm). Solutions of α-CD (55.7 mg), α-CD_2NSc (56.5 mg), β-CD (45.5 mg) and β-CD_2NSc (54.4 mg) in 0.1 M phosphate buffer pH 7.4 (25 ml) were prepared and filtered (0.22 μm) before use.

The column is equilibrated and eluted at 1ml/min with successive concentrations of drug in 0.1 M phosphate buffer (pH 7.4). For each concentration of drug used as eluant a 50 μL sample of pure buffer is run as a blank, followed by 50 μL samples of each of the cyclodextrin solutions. Peaks are detected over the range 300 - 380 nm. The shorter wavelengths are used for increased sensitivity at the lower drug concentrations and the longer wavelengths are used to avoid detector overload at higher drug concentrations. The negative peak which occurs at about 6 minutes corresponds to a depletion of drug in the eluant due to both dilution (calculated from the injection of pure buffer) and inclusion of the drug by the cyclodextrin. The included drug elutes with the cyclodextrin at about 2 minutes and gives rise to a positive peak.

Integration of the negative peak and subtraction of the dilution factor allows the calculation of the number of moles of drug bound per mole of cyclodextrin injected ($\underline{r}$). The value $\underline{r}$ is a measure of the relative abilities of the cyclodextrin derivatives to bind a particular drug.

Measurement of $\underline{r}$ at different concentrations (L) allows the plotting of ($\underline{r}$/L) vs.($\underline{r}$), a Scatchard Plot, for each cyclodextrin studied. The plot should be a straight line with the Y-intercept equal to the binding constant (K).

EXAMPLE 119

Binding constants for Indomethacin with modified Cyclodextrins

cf. J. Chrom. 503, 453 (1990)

The concentrations of indomethacin used to elute the column were 1.003×10^{-4} M, 2.006×10^{-4} M, 4.012×10^{-4} M and 1.003×10^{-3} M and were prepared by dilution of a stock solution of indomethacin (359.0 mg, 1.003×10^{-3} mole) in buffer (1L). All solutions were passed through a 0.22 μm filter before use.

The above procedure gives the following values for the binding of indomethacin at pH 7.4: α-CD, 180; α-CD2NSc, 360; β-CD, 620; β-CD_2NSc, 1900.

EXAMPLE 120

Effect of cyclodextrins on the hydrolysis of N-Benzoyl-(1)-tyrosine ethyl ester (BTEE) by α-Chymotrypsin

N-Benzoyl-(l)-tyrosine ethyl ester (BTEE) is a substrate commonly used to determine the activity of α-chymotrypsin. It is shown that the rate of hydrolysis of BTEE by α-chymotrypsin is slowed in the presence of β-cyclodextrin (β-CD).

Experimental

Enzyme solution. 345 $g.l^{-1}$ Hydrochloric acid (0.1 ml) was made up to 948 ml with Milli-Q® water to give hydrochloric acid-10^{-3} $mol.l^{-1}$. α-Chymotrypsin (from bovine pancreas, type II, Sigma; 15 mg) was dissolved in 10^{-3} $mol.l^{-1}$ hydrochloric acid (100 ml). Buffer Solution. Tris-(hydroxymethyl)-aminomethane (6.055 g) was dissolved in Milli-Q® water to make 250 ml. Calcium chloride dihydrate (10.55 g) was added and the pH of the solution adjusted to 7.8 with concentrated and then dilute hydrochloric acid. The solution was diluted to 1 l with Milli-Q® water and dry methanol (432 ml) added. Substrate solution. BTEE (14.8 mg) was dissolved with sonication in the buffer solution (100 ml). Substrate, β-cyclodextrin solution. Anhydrous β-cyclodextrin (197.4 mg) was dissolved with sonication in the substrate solution above (50 ml). This ensured that the concentration of

BTEE in both solutions was the same.

UV measurements. volumes were measured with a 1000 µl or a 200 µl variable pippetor. Where 3 ml volumes were required 3 x 1000 µl samples were used. Where 150 µl was required a single 150 µl sample was used. All solutions were placed in the spectrophotometer room for three hours before measurements were taken to allow equilibration of the solutions to room temperature. Measurements were made in a matched pair of 1 cm quartz cells the absorption was measured over time at 256 nm.

Control experiment. The reference cell was filled with substrate solution (3 ml) and water (150 µl) and mixed to ensure homogeneity. The sample cell was filled with substrate solution (3 ml) and enzyme solution (150 µl) was added smoothly to the cell. Five seconds after the beginning of the enzymes addition the solution was stirred for 10 seconds. Thirty seconds after the start of the enzymes addition the data collection was started. Data was collected for 10 minutes, by which time the hydrolysis was apparently quite complete. Data were printed out during collection on chart paper and were also collected on disc.

The sample solutions were prepared separately three times and data collected to ensure the reproducibility of the results.

Test experiment. The reference cell was filled with substrate, β-cyclodextrin solution (3 ml) and water (150 µl) and mixed to ensure homogeneity. The sample cell was filled with substrate, β-cyclodextrin solution (3 ml) and enzyme solution (150 µl) was added smoothly to the cell. Five seconds after the beginning of the enzymes addition the solution was stirred for 10 seconds. Thirty seconds after the start of the enzymes addition the data collection was started. Data was collected for 10 minutes, by which time the hydrolysis was apparently quite complete. Data were printed out during collection on chart paper and were also collected on disc.

The sample solutions were prepared separately three times and data collected to ensure the reproducibility of the results.

Discussion and calculations.

Figure 1 shows a typical plot of the experiments. Note the rate of hydrolysis of BTEE in the presence of β-CD is slower than without β-CD.

$[BTEE]_0$ = 14.8 x 10^{-3} g/(313.3 g.mol^{-1} x 0.1 l) x (3 ml/3.15 ml) = 4.49 x 10^{-4} mol.l^{-1}.

$[\beta\text{-CD}]_0$ = 197.4 x 10^{-3} g/(1134.9 g.mol^{-1} x 0.05 l) x (3 ml/3.15 ml) = 3.312-x 10^{-3} mol.l^{-1}.

An arbitrary point was taken near the endpoint of the control reaction and the slope was estimated at this point by eye. At t = 116 seconds the slope was 0.025 units/30 seconds, corresponding to an absorbance (Abs_s) of 0.378. The maximum absorbance (Abs_{max}) for this experiment was 0.414. Abs_{max} corresponds to a N-benzoyl-(*l*)-tyrosine concentration of 4.498 x 10^{-4} mol.l^{-1} or a BTEE concentration of 0 mol.l^{-1}. Abs_{max}-Abs_s is proportional to the [BTEE] at time t, thus: $[BTEE]_s$ = (0.414-0.378)/0.414 x 4.4982 x 10^{-4} mol.l^{-1} = 3.98 x 10^{-5} mol.l^{-1}.

The rate of the enzyme catalysed hydrolysis is proportional to the effective (or available) [BTEE] at concentrations below K_m. In the control experiment the effective [BTEE] equals the total [BTEE], therefore:
$[BTEE]_{eff.s}$ = 3.98 x 10^{-5} mol.l^{-1} corresponding to a slope of 0.025 units/3 seconds.

A point was taken on the test reaction such that the slope matched that found on the control reaction (0.025 units/30 seconds). This corresponded to t = 151 seconds and an absorbance ($Abs_{s.cd}$) of 0.3800. Abs_{max} was 0.4240. The total [BTEE], that is the uncomplexed BTEE available for hydrolysis and that complexed

by β-cyclodextrin, can be calculated from these figures:
$[BTEE]_{s.cd}$ = (0.424-0.380)/0.4240 x 4.498 x 10^{-4} mol.l^{-1} = 4.667 x 10^{-5} mol.l^{-1}.

The uncomplexed BTEE was known from the slope of the graph at this point and was calculated above:
$[BTEE]_{eff.s.cd}$ = 3.980 x 10^{-5} mol.l^{-1}.

The stability constant of the substrate inclusion complex (K) is the ratio of the concentration of the complexed substrate over the product of the concentrations of the free substrate and the free β-cyclodextrin:
K = [β-CD.BTEE]/([β-CD].[BTEE])

$[\beta\text{-CD.BTEE}] = [BTEE]_{s.cd} \cdot [BTEE]_{eff.s.cd}$ = 6.871 x 10^{-6} mol.l^{-1}.
$[\beta\text{-CD}] = [\beta\text{-CD}]_0 - [\beta\text{-CD.BTEE}]$

$[BTEE] = [BTEE]_{eff.s.cd}$

By simple substitution:
K = 6.87 x 10^{-6} mol.l^{-1}/((3.312 x 10^{-3} 6.871 x 10^{-6} mol.l^{-1}) x 3.980 x 10^{-5} mol.l^{-1}) = 52.213

This procedure was repeated several times to gain a measure of error caused by eye. The difference in K was within 10%.

EXAMPLE 121

Piroxicam with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:2 complex

To a solution of β-$CDNH_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Piroxicam (37 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 18 hours during which time a clear yellow solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (258 mg) which was the Piroxicam modified cyclodextrin formulation.

EXAMPLE 122

Piroxicam with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:4 complex

To a solution of β-CDNH$_2$ (500 mg, 0.44 mmol) in Milli-Q® water (20 ml) was added solid Piroxicam (37 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 18 hours during which time a clear yellow solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (508 mg) which was the Piroxicam modified cyclodextrin formulation.

EXAMPLE 123

Indomethacin with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:2 complex

To a solution of β-CDNH$_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Indomethacin (39 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 18 hours during which time a clear yellow solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (265 mg) which was the Indomethacin modified cyclodextrin formulation.

EXAMPLE 124

Indomethacin with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:4 complex

To a solution of β-CDNH$_2$ (500 mg, 0.44 mmol) in Milli-Q® water (20 ml) was added solid Indomethacin (39 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 18 hours during which time a clear yellow solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (515 mg) which was the Indomethacin modified cyclodextrin formulation.

EXAMPLE 125

Naproxen with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:2 complex

To a solution of β-$CDNH_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Naproxen (25 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 2 hours during which time a clear colorless solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (250 mg) which was the Naproxen modified cyclodextrin formulation.

EXAMPLE 126

Naproxen with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:4 complex

To a solution of β-$CDNH_2$ (500 mg, 0.44 mmol) in Milli-Q® water (20 ml) was added solid Naproxen (25 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 2 hours during which time a clear colorless solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (515 mg) which was the Naproxen modified cyclodextrin formulation.

EXAMPLE 127

Ibuprofen with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:2 complex

To a solution of β-$CDNH_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Ibuprofen (22.5 mg, 0.11 mmol) and the resulting suspension was stirred at room temperature for 60 hours during which time a clear colorless solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (270 mg) which was the Ibuprofen modified cyclodextrin formulation.

EXAMPLE 128

Ibuprofen with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:4 complex

To a solution of β-$CDNH_2$ (500 mg, 0.44 mmol) in Milli-Q® water (20 ml) was added solid Ibuprofen (22.5 mg, 0.11 mmol) and

the resulting suspension was stirred at room temperature for 60 hours during which time a clear colorless solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (515 mg) which was the Ibuprofen modified cyclodextrin formulation.

EXAMPLE 129

Piroxicam with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:1 complex

To a solution of β-$CDNH_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Piroxicam (72 mg, 0.219 mmol) and the resulting suspension was stirred at room temperature for 48 hours during which time most but not all of the drug dissolved. The fine suspended solid was removed by filtration and the resulting yellow filtrate was evaporated to dryness *in vacuo*. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (287 mg) which was the Piroxicam modified cyclodextrin formulation.

EXAMPLE 130

Indomethacin with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:1 complex

To a solution of β-$CDNH_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Indomethacin (79 mg, 0.22 mmol) and the resulting suspension was stirred at room temperature for 48 hours during which time most but not all of the drug dissolved. The fine suspended solid was removed by filtration and the resulting yellow filtrate was evaporated to dryness *in vacuo*. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (287 mg) which was the Indomethacin modified cyclodextrin formulation.

EXAMPLE 131

Ibuprofen with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:1 complex

To a solution of β-CDNH$_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Ibuprofen (45 mg, 0.22 mmol) and the resulting suspension was stirred at room temperature for 48 hours during which time most but not all of the drug dissolved. The fine suspended solid was removed by filtration and the resulting yellow filtrate was evaporated to dryness *in vacuo*. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (280 mg) which was the Ibuprofen modified cyclodextrin formulation.

EXAMPLE 132

Naproxen with 6^A-amino-6^A-deoxy-β-cyclodextrin 1:1 complex

To a solution of β-CDNH$_2$ (250 mg, 0.22 mmol) in Milli-Q® water (10 ml) was added solid Naproxen (50 mg, 0.22 mmol) and the resulting suspension was stirred at room temperature for 4 hours during which time a clear colorless solution was obtained. Filtration and evaporation to dryness *in vacuo* resulted in the isolation of a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a yellow powder (268 mg) which was the Naproxen modified cyclodextrin formulation.

EXAMPLE 133

Amiodarone with 6^A-amino-6^A-deoxy-N-(3-carboxypropyl)β-cyclodextrin 1:4 complex

To a solution of β-CDNHCO(CH$_2$)$_2$CO$_2$H.2H$_2$O (147 mg, 0.116 mmol) in Milli-Q® water (40 ml) was added solid Amiodarone (20 mg, 0.029 mmol) and the resulting suspension was stirred at room temperature for 18 hours. The solution was filtered through a 0.2 μ filter and the resultant filtrate was evaporated to dryness *in vacuo* to produce a glassy material. Drying to constant weight *in vacuo* over phosphorus pentoxide gave a colorless powder (138 mg) which was the Amiodarone modified cyclodextrin formulation.

EXAMPLE 134

Amiodarone with 6^A-amino-6^A-deoxy-N-(3-carboxypropyl)-β-cyclodextrin 1:2 complex

Amiodarone.HCl (Sigma, 0.0200 g, 0.0293 mmol) and β-CDNSc (0.0763 g, 0.0618 mmol) were mixed in 40 ml Milli-Q® water and stirred with a magnetic stirrer for 24 hours. The resulting solution contained only a slight haze of undissolved material. The solution was filtered (0.2 μm filter) and dried *in vacuo*. The collected crystals, which were almost transparent, readily dissolved in pure water but not in phosphate buffer of pH 7.

UV spectrascopic determination of the composition of the solid and the solubilities of amiodarone.HCl under various conditions.

Amiodarone.HCl has a maximum absorbance at 242 nm in ethanol, pure water and ethanol/water mixture (4:1 ratio by weight). The extinction coefficient of amiodarone.HCl in ethanol/water mixture solution (4:1 ratio by weight) was measured using a series of solutions of amiodarone.HCl in this solvent. These were prepared by diluting an amiodarone.HCl solution of known concentration in pure ethanol with the appropriate amount of ethanol and water. The extinction coeffecient of amiodarone.HCl at 242 nm in such a mixed solvent is 46,000 (g amiodarone.HCl / g solvent)$^{-1}$.cm^{-1}.

EXAMPLE 135

Solubility of amiodarone.HCl in pure water

The saturated solution of amiodarone.HCl in pure water was prepared by adding an excess of amiodarone.HCl (0.1 g) to 10 ml Milli-Q® water and shaking the sample bottle in a water bath thermostatted at 25.0°C for at least 3 days. The clear solution (0.4 g) was mixed with 1.6 g ethanol. The concentration of the solution was calculated from the measured absorbance at 242 nm and the known extinction coefficient, which led to the determination of the solubility of amiodarone.HCl in pure water, 200 mg.l^{-1}. Same procedure was repeated 3 days after to assure

the saturation of the solution.

EXAMPLE 136

Composition and solubility of the amiodarone/β-CDNSc solid

Anhydrous amiodarone/β-CDNSc complex (0.0364 g) was dissolved in 1.0089 g Milli-Q® water. The resulted solution was very viscous and seemed to be close to its saturation. The solution was diluted 100 fold with water for taking UV spectra. The concentration of the original solution in terms of pure amiodarone.HCl was calculated, using the measured absorbance at 242 nm and the extinction coefficient 46,000 (g amiodarone.HCl/g solvent)$^{-1}$.cm^{-1}, as 6.9 g.l^{-1}, which should present the lower bound of the solubility of amiodarone.HCl in water in the presence of β-CDNSc. It is ~35 times higher than that in pure water without β-CDNSc. Such a concentration can not be achieved by dissolving corresponding amount of amiodarone.HCl and β-CDNSc into water directly.

Subtracting the calculated amount of pure amiodarone.HCl (0.0106 mmole) from the total sample of solid used gave rise to the estimation for the composition of the solid:
amiodarone.HCl : β-CDNSc = 0.0106 mmole : 0.0236 mmole ~ 1 : 2

At 242 nm the extinction coefficient of β-CDNSc is 77 M^{-1}.cm^{-1} and its contribution to the absorbance at 242 nm of the 100 fold diluted amiodarone.HCl/β-CDNSc solution above would be only 0.003, negligibly small compared to the total of ~0.6 absorbance unit.

EXAMPLE 137

In this Example, 6^{A}-amino-6^{A}-deoxy-N-(3-carboxypropyl)-β-cyclodextrin (β-CDNSc) was used to microencapsulate amiodarone for IV injection. Toxicity and mutagenicity also were evaluated and the ability to solubilize amiodarone HCl was also tested *in vitro* and *in vivo*.

Materials and Experimental

Amiodarone HCl was obtained from Sigma chemical Company Ltd. (Dorset, England) and β-CDNSc was prepared as described above. All other chemicals used in the study were of analytical grade.

Pharmacokinetic Study of Amiodarone HCl in Dogs

An appropriate quantity of β-CDNSc was dissolved in water and solid amiodarone HCl was added to give 1:4 (amiodarone:β-CDNSc) molar ratio. The solution was stirred overnight at ambient temperature, filtered, then evaporated to dryness to produce a glassy solid which was dried in a vacuum over solid P_2O_5. The dried glassy solid consisting of the amiodarone HCl/β-CDNSc complex had a solubility of 6.9 of amiodarone HCl per liter of water, which is 35 times higher than that in pure water without β-CDNSc. In this experiment, the complex was dissolved in distilled water to give a clear solution of 4.7 mg amiodarone HCl ml^{-1} and used for subsequent injection. The control formulation was prepared by diluting 6 ml Cordarone X, 300mg (Sanofi Pharmaceuticals, UK) to 20 ml with 5% dextrose solution (equivalent to 15 mg amiodarone HCl ml^{-1}).

Dogs were administered amiodarone HCl as a single injection into the right cephalic vein, using an indwelling catheter, at a dose of 5 mg kg^{-1}. Amiodarone HCl was given with and without β-CDNSc in three periods separated from each other by seven days. Blood (ca 5 ml) was then sampled from either jugular vein of all animals at 0 (predose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours. The blood was collected into heparinized glass vacutainers and centrifuged (1500 g for 10 min. at 18°C) to prepare plasma within 30 min. of collection. Plasma samples were stored at -20°C pending analysis. Plasma concentration of amiodarone HCl in the samples was measured by the high performance liquid chromatography procedure described by Kess et al.

Pharmacokinetic and Statistical Analysis

The peak concentration of amiodarone HCl (Cmax) and the time

at which this occurred (Tmax) were determined. The area under the curve of plasma concentration plotted against time after administration (AUC) was calculated by the linear trapezoidal rule of the interval 0-24 h and 0-infinity. The half life of amiodarone HCl in plasma was determined from the terminal, linear phase of the amiodarone HCl elimination curve. All the variables except Cmax were subject to an analysis of variance (ANOVA) including terms for group, subject (group), formulation and phase effects. Following each ANOVA, pairwise comparison of each test formulation against the control formulation were made using t-test each at a 5% significant level (two-tailed). Ninety-five percent confidence intervals were calculated for the difference between each test formulation and the control formulation.

Results

Before experiments, hepatic and renal functions of all dogs were found to be within the normal range for this species. Following IV administration of Cordarone X, all dogs appeared very groggy and lethargic in the first hour. Summary of side effects observed is shown in Table 8. Animals given amiodarone HCl-β-CDNSc did not display any adverse effects.

Table 8. Side effects observed in dogs receiving Cordarone X intravenous injection.

Dog	During dosing	Post Dose
7	-------	lethargy, slow deep breathing
8	agitated	lethargy, slow deep breathing
9	agitated	lethargy, deep breathing, vomiting
10	agitated	lethargy, increased respiratory rate
11	agitated	lethargy, slow deep breathing
12	unsteady on legs, rapid breathing	soft faeces

When the plasma concentration-time were plotted semilogarithmically (Figure 9), it become apparent that plasma concentration particularly at the distribution phase with higher in the control dogs 10 and 12 in that group also displayed

erratic fluctuation of plasma drug levels in the first four hours after injection (Figures 10 and 11). Such phenomenon was not observed in animals treated with amiodarone HCl-β-CDNSc.

The pharmacokinetic parameters for amiodarone HCl are given in Table 9. Significant differences were found in AUC (0-24) and Cmax ($p=0.02$ and $p=0.05$ respectively). The differences reflected significant increase in the volumes of distribution in the dogs treated with amiodarone HCl-β-CDNSc. No significant difference, however, is found in elimination half-life, Tmax and AUC (0-infinity). The wide individual variation in these pharmacokinetic parameters may prevent any significant difference, if present, between these two groups to be discerned.

Table 9. Pharmacokinetic parameters for Amiodarone HCl - mean (SD).

Parameters	Amiodarone	Amiodarone-β-CDNSc	Significance
AUC (0-24 h)	4.445 (2.118)	2.684 (0.476)	0.02
AUC (0-infinity)	5.982 (3.519)	5.464 (2.991)	NS
Cmax (μg/ml)	2.100 (1.403)	0.806 (0.193)	0.05
Tmax (h)	0.458 (0.51)	0.250 (0)	NS
half-life (h)	17.646 (14.04)	36.264 (32.332)	NS

Discussion

As described in other studies, after intravenous bolus administration, plasma amiodarone levels fell substantially in the first 10 to 15 minutes and then declined slowly with a long elimination half-life.

Wide intersubject variation in the pharmacokinetic parameters such as Cmax, AUC (0-24), and Tmax was observed in the control group (Table 9). Such variation was found to be reduced in the group receiving the amiodarone-β-CDNSc injection. This

improvement in the pharmacokinetic profile of the drug may allow a more accurate prediction of drug levels from the doses administered and amiodarone therapy for patients can be individually tailored.

When amiodarone microencapsulated with β-CDNSc was injected, AUC (0-24) and Cmax were increased, while no significant changes were found in the AUC (0-infinity) and elimination half-life. This indicated that the volumes of distribution particularly at the early phase of distribution was increased. The increase was possibly caused by the improvement in dissolution of amiodarone by β-CDNSc. The major finding of this study is that CDNSc eliminated the common side effects observed after intravenous injection of amiodarone. The side effects appeared after rapid intravenous injection of amiodarone relates to the effects of diluting the solubilizing agent - polysorbate 80 and subsequent precipitation of the compound. β-CDNSc may thus be a more suitable solubilizing agent than polysorbate 80.

The plasma disappearance curves of dogs 10 and 12 in the control phase had peaks appear in the first four hours after injection. Others have also observed secondary peaks between 4 and 12 hours after intravenous administration, which were tentatively attributed to enterohepatic circulation of amiodarone. However, the substantial rise and fall of plasma levels observed in these two dogs may be more related to the dissolution problems of amiodarone of the original formulation.

EXAMPLE 138

Stability Constants for the inclusion of Cimetidine by β-Cyclodextrin and 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-β-cyclodextrin

A series of solutions of cimetidine (8.4×10^{-4} M, 4.2×10^{-4} M, 21×10^{-4} M and 1.05×10^{-4} M) were prepared by dilution of a stock solution of cimetidine (529 mg) in 0.1 M phosphate buffer, pH 7.4. Solutions of β-cyclodextrin (54 mg) and 6^A-amino-6^A-

deoxy-6^A-N-(3-carboxypropanoyl)-ß-cyclodextrin (67 mg) in buffer were prepared and all solutions were filtered (0.22 µm). A Merck Lichrosorb Diol column (4 x 250 mm) was equilibrated with successive concentrations of the drug solutions at 1 ml/min and the eluent was monitored over the range 235-255 nm (lower wavelengths for lower drug concentrations) using a Waters Lambda-Max 481 UV detector. For each concentration of Cimetidine used as eluent a 50 µl sample of pure buffer was run as a blank, followed by 50 µl samples of each of the cyclodextrin solutions, injected via an autosampler. The negative peak which occurs at about 6 minutes corresponds to a depletion of Cimetidine in the eluent due to both dilution (calculated from the injection of pure buffer) and the inclusion of cimetidine by the cyclodextrin. The included cimetidine elutes with the cyclodextrin at about 2 minutes and gives rise to a positive peak. Integration of the negative peak and subtraction of the dilution factor allows the calculation of the number of moles of cimetidine bound per mole of the cyclodextrin injected. These measurements at different drug concentrations allow the calculation of the stability constants for the inclusion complexes (K). Thus K = 40 M^{-1} s^{-1} for the complexation of the cimetidine by ß-cyclodextrin while K = 300 M^{-1} s^{-1} for the complexation of cimetidine by 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-ß-cyclodextrin.

EXAMPLE 139

Preparation and Water Solubility of a 1:1 Formulation of cimetidine with 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-ß-cyclodextrin

Cimetidine (0.08 g) and 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-ß-cyclodextrin (0.43g) were dissolved in water (10ml). The solution was evaporated to dryness under reduced pressure and the resulting solid was dried in a dessicator over P_2O_5. The 1:1 formulation of cimetidine with 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-ß-cyclodextrin prepared in this way dissolved completely in water (0.5ml) to give a clear solution.

It will be apparent to those skilled in the art that various modifications and variations can be made to the compositions of matter and methods of this invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents. All references in the claims to a composition of matter such as an inclusion complex, cyclodextrin derivative, intermediate, linked cyclodextrins, prodrug, composition, a process reactant, and pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic or personal care agent, whether in general or by name, expressly includes the salts and hydrates thereof.

Thus, one of ordinary skill will recognize many uses for cyclodextrin derivatives and inclusion complexes in accordance with this invention. Such uses can include therapeutic and diagnostic drug delivery, such as multiple routes of administration (i.v., p.o., ophthalmic, transdermal, etc.), improved bioavailability, reduction of irritating drug effects, quantitative reliability, reduced dosing volume, elimination of organic solvents, stable, convenient storage and handling, and previously insoluble or unstable drugs may now be considered for development which enhances removal of lipophilic substances from blood.

Uses for the diagnostic kits include improved low end sensitivity, reduced reaction times, more stable liquid components, greater recovered bioactivity in lyophilized components, reduced effect of interfering substances in serum, plasma and urine specimens and enhanced spectrophotometric response.

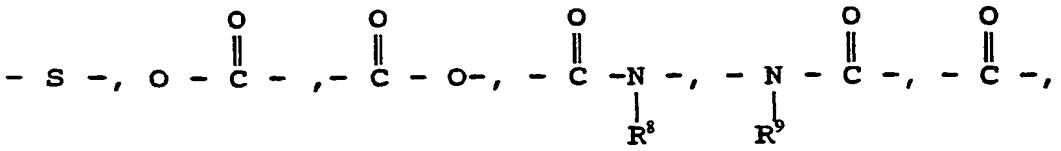
With regard to separation sciences, the following applies: enantiomeric separations via liquid chromatography, preparative chromatography, improved electrophoretic, iontophoretic and isotachophoretic methods.

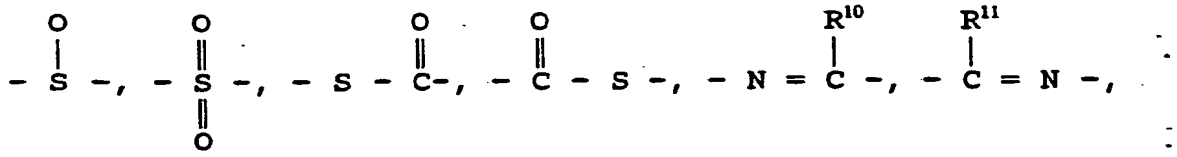
Bioprocessing enables improved down-line recovery (quantity), improved bioactivity (quality), and greater handling convenience. Also, with regard to cell culture, serum free formulations and enhanced productivity of enzymatic reactions are provided.

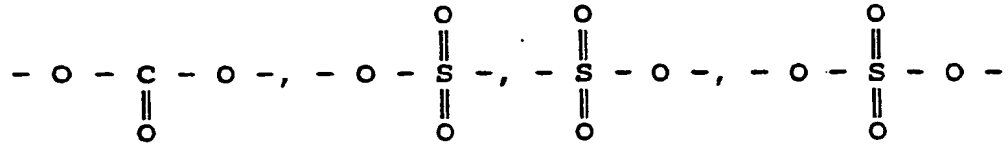
This invention is also applicable to food products, cosmetics, toiletries, taste/smell masking, stable, convenient forms, timed release, homogeneous reaction mixtures, catalyzed reactions, and greater precision and reproducibility of laborator techniques.

WHAT IS CLAIMED IS:

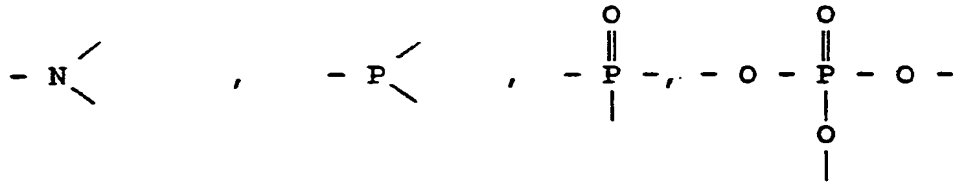
1. A cyclodextrin derivative comprising an otherwise substituted or unsubstituted cyclodextrin in which at least one C2, C3 or C6 hydroxyl is substituted with a group selected from -XR^1, -YR^2R^3, -$SiR^4R^5R^6$, and -R^7, wherein X can represent







Y can represent



and wherein R^1 to R^{11} can each represent the same or different groups selected from: the groups -XR^1, -YR^2R^3, -$SiR^4R^5R^6$, and -R^7 are as defined above, hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl, heterocyclyl, and wherein any two or three groups bonded to the same substituent can be taken together to represent a single group multiply bonded to said same substituent, and wherein R^1 to R^{11} may be further substituted by at least one -XR^1, -YR^2R^3, -$SiR^4R^5R^6$, -R^7, halogen, and OR^{12}, wherein R^{12} is as defined for R^1 to R^{11}, and

wherein when said cyclodextrin comprises at least one substitution as describe above, or is substituted solely for one or more hydroxyls selected from the group of C2 and C3 hydroxyls, said cyclodextrin may also be an substituted by an ether group R^{12}-O-, wherein R^{12} is as defined above for R^1 to R^{11}.

2. A cyclodextrin derivative according to claim 1, wherein said at least one substitution is of the formula $-YR^2R^3$, wherein Y is N, and R^2 and R^3 are as previously defined.

3. A cyclodextrin derivative according to claim 2, wherein R^2 is hydrogen and R^3 represents amino, hydroxyl, carboxyl, sulfonate (SO_3^-), phosphate (PO_4^{-3}), substituted alkyl, cycloalkyl, or aryl, or R^2 and R^3 are taken together to represent a hereto substituted multiply bonded alkyl group.

4. A cyclodextrin derivative according to claim 1, having the formula CD - W - R^{13} - L, wherein

CD represents an otherwise substituted or unsubstituted cyclodextrin,

W represents a functional linking group,

R^{13} represents a group defined the same as R^1-R^{12} above, and

L represents a group selected from reactive, charged, polar or associating groups selected from amino, carboxyl, hydroxyl, sulfonate, phosphate, acyloxy, alkyloxy and thiyl.

5. A cyclodextrin derivative according to claim 4, wherein

W represents amino, amide, ester, thioether, thioamide, thioester or thiol,

R^{13} represents an otherwise substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl, and

L represents a carboxyl group.

6. A cyclodextrin derivative according to claim 5, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-3, 1-6, 1-10, 10-

20 and greater than 20 carbons.

7. A cyclodextrin derivative according to claim 6, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-3 carbons.

8. A cyclodextrin derivative according to claim 6, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-6 carbons.

9. A cyclodextrin derivative according to claim 6, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-10 carbons.

10. A cyclodextrin derivative according to claim 7, comprising a 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl) cyclodextrin.

11. A cyclodextrin derivative according to claim 10, which is a β-cyclodextrin.

12. A cyclodextrin derivative according to claim 4, wherein L represents a group having a net negative charge.

13. A cyclodextrin derivative according to claim 12, wherein L represents a group selected from hydroxyL, carboxyl, phosphate (PO_4^{-3}) or sulfonate (SO_3^{-1}).

14. A cyclodextrin derivative according to claim 13, wherein R represents an otherwise substituted or unsubstituted alkyl group selected from the group of alkyls having from 1-3, 1-6, 1-10, 10-20 and greater than 20 carbons.

15. A cyclodextrin derivative according to claim 14, wherein R represents an otherwise substituted or unsubstituted alkyl group selected from the group of alkyls having from 1-3, 1-6 and 1-10 carbons.

16. A cyclodextrin derivative according to any of claims 4-15, wherein L is amino, ester or amide.

17. A cyclodextrin derivative according to claim 1, having the formula CD - X - R^{14} - Q, wherein:

X represents $-N(R^{15})-C(=O)-$ or $-S-C(=O)-$

R^{14} and R^{15} represent groups as defined by R^1-R^{13} above, and

Q is a carboxylic acid group or a carboxylic acid group derivatized to undergo substitution.

18. A cyclodextrin derivative according to claim 17, wherein R^{14} represents an otherwise substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl, and

Q is a carboxylic acid group derivatized to undergo substitution.

19. A cyclodextrin derivative according to claim 18, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-3, 1-6, 1-10, 10-20 and greater than 20 carbons.

20. A cyclodextrin derivative according to claim 19, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-3 carbons.

21. A cyclodextrin derivative according to claim 19, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-6 carbons.

22. A cyclodextrin derivative according to claim 19, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-10 carbons.

23. A cyclodextrin derivative according to claim 20, comprising the acid chloride, acid anhydride or ester of a 6^A-amino-6^A-deoxy-6^A-N-(3-carboxypropanoyl)-cyclodextrin.

24. A cyclodextrin derivative according to any of claims 18-23, wherein Q is an acid chloride, acid anhydride or ester.

25. A cyclodextrin derivative according to any of claims 1-10, 12-15 and 17-23, wherein said cyclodextrin is a β-cyclodextrin.

26. A cyclodextrin derivative according to claim 24, wherein said cyclodextrin is a β-cyclodextrin.

27. A cyclodextrin derivative according to any of claims 1-10, 12-15 and 17-23, wherein said cyclodextrin is an α-, γ- or δ-cyclodextrin.

28. A cyclodextrin derivative according to claim 24, wherein said cyclodextrin is an α-, γ- or δ-cyclodextrin.

29. An inclusion complex comprising at least one pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic, personal care or other useful agent included in a cyclodextrin derivative according to any of claims 1-28.

30. An inclusion complex according to claim 29, wherein said agent is a pharmaceutical.

31. An inclusion complex according to claim 30, wherein said agent is cimetidine.

32. An inclusion complex according to claim 30, wherein said agent is a polypeptide.

33. An inclusion complex according to claim 30, wherein said agent is amiodarone or piroxicam.

34. A cyclodextrin derivative comprising first and second otherwise substituted or unsubstituted cyclodextrins covalently bonded together by at least one linking group which links a C6 carbon on the first cyclodextrin to a C2 or C3 carbon on the second cyclodextrin.

35. A cyclodextrin derivative according to claim 34, wherein the first and second cyclodextrins are linked by at least one linking group of the formula - X - R^{16} - Y - or - R^{17} - , wherein

X and Y can be the same or different, and represent functional linking groups, and

R^{16} and R^{17} represent groups as defined by R^1-R^{15} above.

36. A cyclodextrin derivative according to claim 35, wherein the linking group is of the formula - X - R^{16} - Y - , and X and Y represent ether, thiol, thioether, ester, thioester, amide, thioamide, or amine, and

R^{16} represents substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl.

37. A cyclodextrin derivative according to claim 36, wherein X and Y represent amide or thioamide, and R^{16} represents substituted or unsubstituted alkyl.

38. A cyclodextrin derivative according to any of claims 34-37, wherein said first and second cyclodextrins are different and are selected from α-, β-, γ- or δ-cyclodextrin.

39. A cyclodextrin derivative according to any of claims 34-38, wherein said first and second cyclodextrins are differently substituted.

40. A cyclodextrin derivative according to any of claims 34-39, wherein said linking group links said second cyclodextrin at a C3 carbon.

41. A cyclodextrin derivative comprising first and second otherwise substituted or unsubstituted cyclodextrins covalently bonded together by at least one linking group which links a C2, C3 or C6 carbon on the first cyclodextrin to a C2, C3 or C6 carbon on the second cyclodextrin, and wherein said first and second cylodextrins are different and are selected from α-, β-, γ- or δ-cyclodextrin.

42. A cyclodextrin derivative according to claim 41, wherein the first and second cyclodextrins are linked by at least one linking group of the formula - X - R^{16} - Y - or - R^{17} - , wherein

X and Y can be the same or different, and represent functional linking groups, and

R^{16} and R^{17} represent groups as defined by R^1-R^{15} above.

43. A cyclodextrin derivative according to claim 42, wherein the linking group is of the formula - X - R^{16} - Y - , and X and Y represent ether, thiol, thioether, ester, thioester, amide, thioamide, or amine, and

R^{16} represents substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl.

44. A cyclodextrin derivative according to claim 43, wherein X and Y represent amide or thioamide, and R^{16} represents substituted or unsubstituted alkyl.

45. A cyclodextrin derivative according to any of claims 41-44, wherein said linking group links said first cyclodextrin at a C6 carbon to said second cyclodextrin at a C3 carbon.

46. A cyclodextrin derivative according to any of claims 41-45, wherein said first and second cyclodextrins are different and are selected from α-, β-, γ- or δ-cyclodextrin.

47. A cyclodextrin derivative comprising first and second otherwise substituted or unsubstituted cyclodextrins covalently bonded together by at least one linking group which links a C2, C3 or C6 carbon on the first cyclodextrin to a C2, C3 or C6 carbon on the second cyclodextrin, and wherein said first and second cylodextrins are differently substituted.

48. A cyclodextrin derivative according to claim 47, wherein the first and second cyclodextrins are linked by at least one linking group of the formula - X - R^{16} - Y - or - R^{17} - , wherein

X and Y can be the same or different, and represent functional linking groups, and

R^{16} and R^{17} represent groups as defined by R^1-R^{15} above.

49. A cyclodextrin derivative according to claim 48, wherein the linking group is of the formula - X - R^{16} - Y - , and X and Y represent ether, thiol, thioether, ester, thioester, amide, thioamide, or amine, and

R^{16} represents substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl.

50. A cyclodextrin derivative according to claim 49, wherein X and Y represent amide or thioamide, and R^{16} represents substituted or unsubstituted alkyl.

51. A cyclodextrin derivative according to any of claims 47-50, wherein said linking group links said first cyclodextrin at a C6 carbon to said second cyclodextrin at a C3 carbon.

52. An inclusion complex comprising at least one pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic, personal care or other useful agent included in a cyclodextrin derivative according to any of claims 29-51.

53. A cyclodextrin derivative comprising two cyclodextrins covalently bonded to each other by at least one linking group, wherein said at least one linking group links a first cyclodextrin at a C2, C3 or C6 position to a second cyclodextrin at a C2, C3 or C6 position, and wherein said two cyclodextrins may be further substituted, and wherein when said cyclodextrins are not otherwise substituted and are linked by only one linking group, said linking group is other than a disulfide that links the two cyclodextrins at the C6 positions of each cyclodextrin.

54. A cyclodextrin derivative according to claim 53, wherein the first and second cyclodextrins are linked by at least one linking group of the formula - X - R^{16} - Y - or - R^{17} - , wherein

X and Y can be the same or different, and represent functional linking groups, and

R^{16} and R^{17} represent groups as defined by R^1-R^{15} above.

55. A cyclodextrin derivative according to claim 54, wherein the linking group is of the formula - X - R^{16} - Y - , and X and Y represent ether, thiol, thioether, ester, thioester, amide, thioamide, or amine, and

R^{16} represents substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl.

56. A cyclodextrin derivative according to claim 55, wherein X and Y represent amide or thioamide, and R^{16} represents substituted or unsubstituted alkyl.

57. A cyclodextrin derivative according to any of claims 53-56, wherein said linking group links said first cyclodextrin at a C6 carbon to said second cyclodextrin at a C3 carbon.

58. A cyclodextrin derivative according to any of claims 53-56, wherein each of said two cyclodextrins has one or more

substituents which can associate with one or more substituents on the other cyclodextrin.

59. A cyclodextrin derivative according to claim 58, wherein said associable groups comprise charged or polar atoms or groups of atoms.

60. A cyclodextrin derivative according to claim 59, wherein said associable groups are oppositely charged in the acidic environment of the stomach.

61. A cyclodextrin derivative according to claim 60, wherein said associable groups comprise amino, hydroxyl, carboxyl, sulfonate (SO_3^-) or phosphate (PO_4^{-3}).

62. A cyclodextrin derivative according to claim 58, wherein said associable groups lose charge or take on opposite charges in the relatively neutral environment of the intestines.

63. A cyclodextrin derivative according to claim 62, wherein said associable groups comprise histidine or imidazole.

64. An inclusion complex comprising at least one pharmaceutical, pesticidal, herbicidal, agricultural, cosmetic, personal care or other useful agent included in a cyclodextrin derivative according to any of claims 1-30, 34-51 and 53-63.

65. An inclusion complex according to claim 64, wherein said agent is a pharmaceutical.

66. An inclusion complex according to claim 65, wherein said agent is cimetidine.

67. An inclusion complex according to claim 65, wherein said agent is a polypeptide.

68. An inclusion complex according to claim 65, wherein said agent is amiodarone or piroxicam.

69. A cyclodextrin derivative comprising an otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins, which are covalently bound to a useful agent wherein the covalent bond, when broken, will yield the agent in an active form.

70. A cyclodextrin derivative according to claim 69, wherein said derivative comprises a pharmaceutical agent and the covalent bond will be broken down in the normally occurring internal activity of a host animal.

71. A cyclodextrin derivative according to claim 69, wherein said covalent bond can be broken by hydrolysis.

72. A cyclodextrin derivative according to claim 69, wherein said covalent bond is an ester or amide.

72. A cyclodextrin derivative comprising an otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins covalently bonded to a carrier which can target cells of interest within a patient.

73. A cyclodextrin derivative according to claim 72, wherein said carrier comprises an antibody or fragment thereof, hormones or lymphokines.

74. A cyclodextrin derivative according to claim 73, wherein said carrier is an antibody or fragment thereof.

75. A cyclodextrin derivative according to claim 73, wherein said antibody or fragment thereof is bonded to the

cyclodextrin through an ester, amide, thioester, thioamide or other sulfur-containing bond.

76. A composition comprising an otherwise substituted or unsubstituted cyclodextrin, or two or more otherwise substituted or unsubstituted linked cyclodextrins covalently bonded, physically entrapped or encapsulated, or otherwise associated with a carrier useful for localized or prolonged delivery of a useful agent.

77. A composition according to claim 76, wherein said cyclodextrin or linked cyclodextrins are covalently bonded to said carrier.

78. A composition according to claim 76, wherein said cyclodextrin or linked cyclodextrins are entrapped, encapsulated or otherwise associated with said carrier.

79. A composition according to claim 78, wherein said carrier is a liposome.

80. A composition according to any of claims 76-79, wherein said useful agent is a pharmaceutical agent which is included in said cyclodextrin or linked cyclodextrins.

81. A composition according to any of claims 76-79, wherein said useful agent is a pharmaceutical agent which is covalently bonded to said cyclodextrin or linked cyclodextrins such that the covalent bond, when broken, yields the pharmaceutical in an active form.

82. A pharmaceutical composition comprising a cyclodextrin derivative according to any of claims 1-74, which is in form suitable for oral delivery.

83. A pharmaceutical composition according to claim 82, which is in the form of an aqueous solution.

84. A pharmaceutical composition according to claim 82, which is in the form of a chewable tablet.

85. A pharmaceutical composition according to claim 82, which is in the form of an effervescent tablet.

86. A pharmaceutical composition comprising a cyclodextrin derivative according to any of claims 1-74, which is in form suitable for parenteral delivery.

87. A pharmaceutical composition comprising a cyclodextrin derivative according to any of claims 1-74, which is in form suitable for surgical insertion into a patient.

88. A process for designing cyclodextrin inclusion complexes comprising a useful agent and an otherwise substituted or unsubstituted cyclodextrin or two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, said process comprising:

A. determining whether said useful agent possesses at least one first group capable of non-covalent association with at least one second group;

B. determining the orientation of the agent in the cyclodextrin annulus and the relative position of the at least one first group by considering the dipole moment of the agent and the position of hydrophobic or apolar groups on said agent; and

C. selectively substituting at least one substituent comprising said at least one second group onto one or more C2, C3 or C6 positions of said cyclodextrin or said linked cyclodextrins, said at least one substituent being configured in order to position said second group in the approximate vicinity of said first group to promote association therebetween.

89. A process for designing cyclodextrin inclusion complexes according to claim 88, wherein said first and second groups are charged, polar or capable of hydrogen bonding.

90. A process for designing cyclodextrin inclusion complexes according to claim 89, wherein said first and second groups are selected from the group consisting of amino, hydroxyl, carboxyl, sulfonate (SO_3^-), phosphate (PO_4^{-3}), acyloxy, alkyloxy or thiyl.

91. A process for designing cyclodextrin inclusion complexes according to any of claims 88-91, wherein said inclusion complex comprises two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, and wherein said process further comprises the steps of;

A. determining the dipole moment of each individual cyclodextrin of the linked cyclodextrins; and

B. linking the cyclodextrins such that the dipole moments align where possible.

92. A process for designing cyclodextrin inclusion complexes according to claim 91, wherein only two cyclodextrins are linked, and the C6 carbon of one cyclodextrin is linked to a C2 or C3 carbon of the other cyclodextrin.

93. A process for designing cyclodextrin inclusion complexes according to claim 92, wherein only two cyclodextrins are linked, and the C6 carbon of one cyclodextrin is linked to a C3 carbon of the other cyclodextrin.

94. A process for masking the taste of a pharmaceutical agent comprising the step of forming an inclusion complex comprising said agent and a cyclodextrin derivative according to any of claims 1-30, 34-51 and 53-63.

95. A process according to claim 94, wherein the pharmaceutical agent is cimetidine.

96. A process according to claim 95, wherein the cyclodextrin derivative is according to claim 5.

97. A process according to claim 95, wherein the cyclodextrin derivative is according to claim 8.

98. A process for masking the taste of a pharmaceutical agent comprising the step of forming a cyclodextrin derivative according to any of claims 70-72.

99. A process for increasing the solubility of a pharmaceutical or other useful agent comprising the step of forming an inclusion complex comprising said agent and a cyclodextrin derivative according to any of claims 1-30, 34-51 and 53-63.

100. A process according to claim 99, wherein the pharmaceutical agent is cimetidine.

101. A process according to claim 99, wherein the cyclodextrin derivative is according to claim 5.

102. A process according to claim 99, wherein the cyclodextrin derivative is according to claim 8.

103. A process for masking the taste of a pharmaceutical agent comprising the step of forming a cyclodextrin derivative according to any of claims 70-72.

104. A process for targeting a pharmaceutical agent to a selected group of cells, comprising the steps of:

A. conjugating an otherwise substituted or unsubstituted cyclodextrin or two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, to a carrier capable of targeting a selected group of cells;

B. forming an inclusion complex between the substituted or unsubstituted cyclodextrin of the conjugant and the pharmaceutical agent; and

C. placing the inclusion complex formed in Step B into proximity of said selected group of cells.

105. A process according to claim 104, wherein said selected group of cells is *in vivo*.

106. A process according to claim 105, wherein said carrier comprises a liposome, an antibody or fragment thereof, a hormone or a lymphokine.

107. A process according to claim 106, wherein said carrier comprises an antibody or fragment thereof.

108. A process according to claim 107, wherein said agent comprises an antineoplastic agent or a diagnostic agent.

109. A process for encapsulating into a liposome a pharmaceutical agent of relatively low solubility or wettability, comprising the steps of:

A. forming an inclusion complex between a cyclodextrin derivative comprising a substituted or unsubstituted cyclodextrin or two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, and the agent and the pharmaceutical agent; and

B. encapsulating the inclusion complex formed in Step A into a liposome.

110. A process according to claim 109, wherein said cyclodextrin derivative is according to any of claims 1-30, 34-51 and 53-63.

111. A process for improving the stability of a pharmaceutical agent that is encapsulated in a liposome, comprising the steps of:

A. forming an inclusion complex between a cyclodextrin derivative comprising a substituted or unsubstituted cyclodextrin or two or more otherwise substituted or

unsubstituted cyclodextrins linked by at least one linking group, and the pharmaceutical agent; and

B. encapsulating the inclusion complex formed in Step A into a liposome.

112. A process according to claim 109, wherein said cyclodextrin derivative is according to any of claims 1-30, 34-51 and 53-63.

113. A process for improving the stability of a pharmaceutical agent that is encapsulated in a liposome, comprising the steps of:

A. forming a cyclodextrin derivative in which a pharmaceutical agent is covalently bound to a otherwise substituted or unsubstituted cyclodextrin or to two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, wherein the bond, when broken, will yield the agent in an active form; and

B. encapsulating the cyclodextrin derivative formed in Step A into a liposome.

114. A process according to claim 113, wherein said cyclodextrin derivative is according to any of claims 70-72.

115. A process for decreasing or preventing an immunogenic reaction of a patient to a pharmaceutical agent comprising the steps of:

A. forming an inclusion complex between a cyclodextrin derivative comprising a substituted or unsubstituted cyclodextrin or two or more otherwise substituted or unsubstituted cyclodextrins linked by at least one linking group, and the pharmaceutical agent; and

B. parenterally administering a pharmaceutical comprising the inclusion composition formed in Step A.

116. A process according to claim 115, wherein said cyclodextrin derivative is according to any of claims 1-30, 34-51 and 53-63.

117. A process for the treatment of metabolic disorders associated with compounds produced *in vivo* through enzyme catalysis, comprising administering to a patient in need of treatment a therapeutically effective amount of a substituted or unsubstituted cyclodextrin.

118. A process according to claim 117, wherein said disorder results from the over-production of a physiologically active compound.

119. A process according to claim 118, wherein said disorder is epilepsy.

120. A process according to claim 118, wherein said disorder is associated with the *in vivo* production of the catecholamine neurotransmitters, noradrenaline and adrenaline, from tyrosine.

121. A process for the treatment of a patient suffering from an excess of a toxin comprising administering to the patient in a therapeutically effective amount of a substituted or unsubstituted cyclodextrin.

122. A process according to any of claims 117-122, wherein said cyclodextrin derivative is according to any of claims 1-30, 34-51 and 53-63.

123. A process for making a cyclodextrin derivative of the formula CD - X - R^{14} - Q, wherein:

X represents $-N(R^{15})-C(=O)-$ or $-S-C(=O)-$

R^{14} and R^{15} represent groups as defined by R^1-R^{13} above, and

Q is a carboxylic acid group or a carboxylic acid group

derivatized to undergo substitution, comprising the steps of: A reacting a compound of the formula

$$CD - \underset{\underset{R^{15}}{|}}{N} - H \quad \text{or} \quad CD - S - H$$

wherein R^{15} is as defined above, with a dicarboxylic acid of the formula $Q - R^{14} - Q$ which has been derivatized to undergo substitution, and

B. isolating the product.

124. A process according to claim 123, wherein the cyclodextrin derivative of is reacted with the derivatized dicarboxylic acid in a molar ratio of about 1:1 or less.

125. A process according to claim 123 or 124, wherein the cyclodextrin derivative of is reacted with the derivatized dicarboxylic acid for about three hours or less.

126. A process according to any of claims 123-125, wherein R^{14} represents an otherwise substituted or unsubstituted: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, aryl, heteroaryl and heterocyclyl.

127. A process according to claim 126, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-3, 1-6, 1-10, 10-20 and greater than 20 carbons.

128. A process according to claim 126, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-3 carbons.

129. A process according to claim 126, wherein said otherwise substituted or unsubstituted alkyl group is selected from the group of alkyls having from 1-6 carbons.

130. A process according to claim 126, wherein said otherwise substituted or unsubstituted alkyl group is selected

from the group of alkyls having from 1-10 carbons.

131. A process according to claim 128, comprising the acid chloride, acid anhydride or ester of a 6^-amino-6^-deoxy-6^-N-(3-carboxypropanoyl) cyclodextrin.

132. A process according to any of claims 124-131, wherein said cyclodextrin is a β-cyclodextrin.

133. A process according to any of claims 124-131 said cyclodextrin is an α-, γ- or δ-cyclodextrin.

134. A process according to any of claims 123-125, wherein said carboxyl groups when derivatized can be the same or different and are selected from acid chloride, acid anhydride and ester.

135. A process for preparing linked cyclodextrins of the formula $CD1 - X - R^{14} - Y - CD^2$, wherein

CD1 and CD^2 may be the same or different and are selected from otherwise substituted or unsubstituted cyclodextrins,

X represents $-\underset{\underset{R^{15}}{|}}{N}-\overset{\overset{O}{\|}}{C}-$ or $-S-\overset{\overset{O}{\|}}{C}-$,

Y represents $-\overset{\overset{O}{\|}}{C}-\underset{\underset{R^{18}}{|}}{N}$ or $-\overset{\overset{O}{\|}}{C}-S-$, and

R^{14}, R^{15}, and R^{18} represent groups as defined by R^1-R^{13} above, wherein said process comprises the steps of:

A. reacting a cyclodextrin derivative of the formula $CD^1 - X - R^{14} - Q$, wherein Q is a carboxylic acid group or a carboxylic acid group derivatized to undergo substitution, with a compound of the formula $CD^2 - \underset{\underset{R^{18}}{|}}{N} - H$ or $CD^2 - S - H$, and

B. isolating the product.

136. A process according to claim 135, wherein CD^1 - X - and CD^2 - Y - are different.

137. A process according to claim 135, wherein a C6 carbon of CD^1 is linked to a C2 or C3 carbon of CD^2.

138. A process according to claim 135, wherein a C6 carbon of CD^1 is linked to a C3 carbon of CD^2.

139. A process according to claim 135, wherein CD^1 and CD^2 are different cyclodextrins, and are selected from the group consisting of α-, β-, γ- or δ-cyclodextrin.

FIG. 1

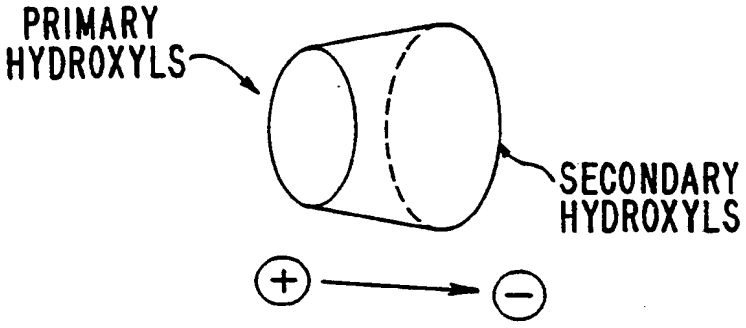


FIG. 2

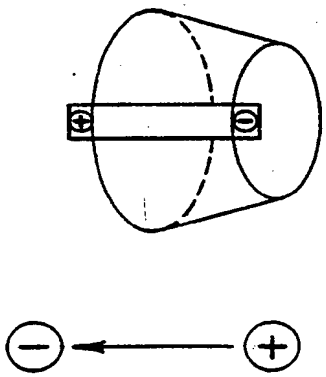
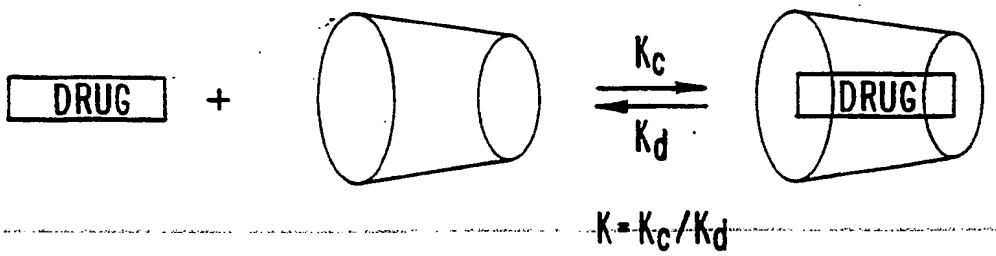


FIG. 3



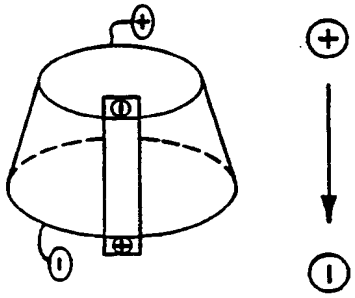


FIG. 4

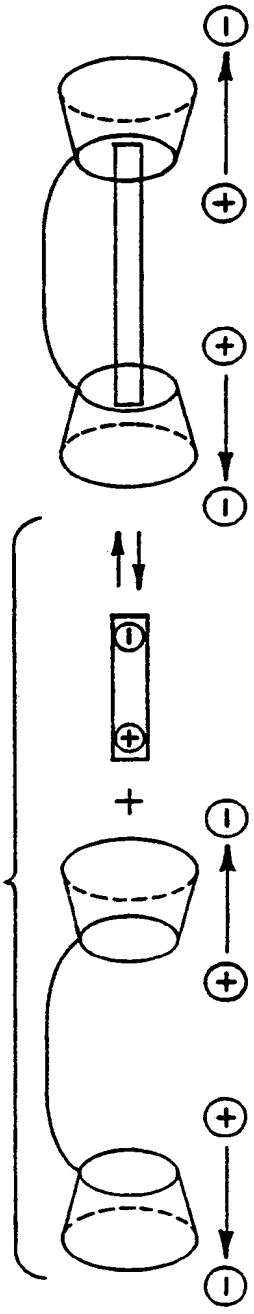


FIG. 5A

FIG. 5C

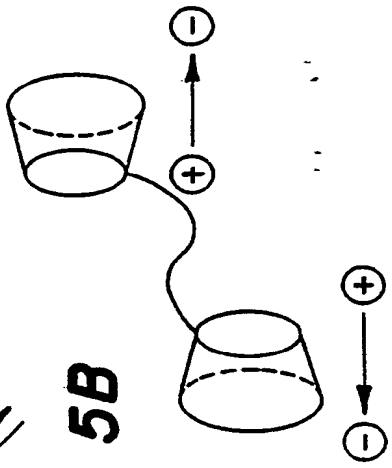


FIG. 5B

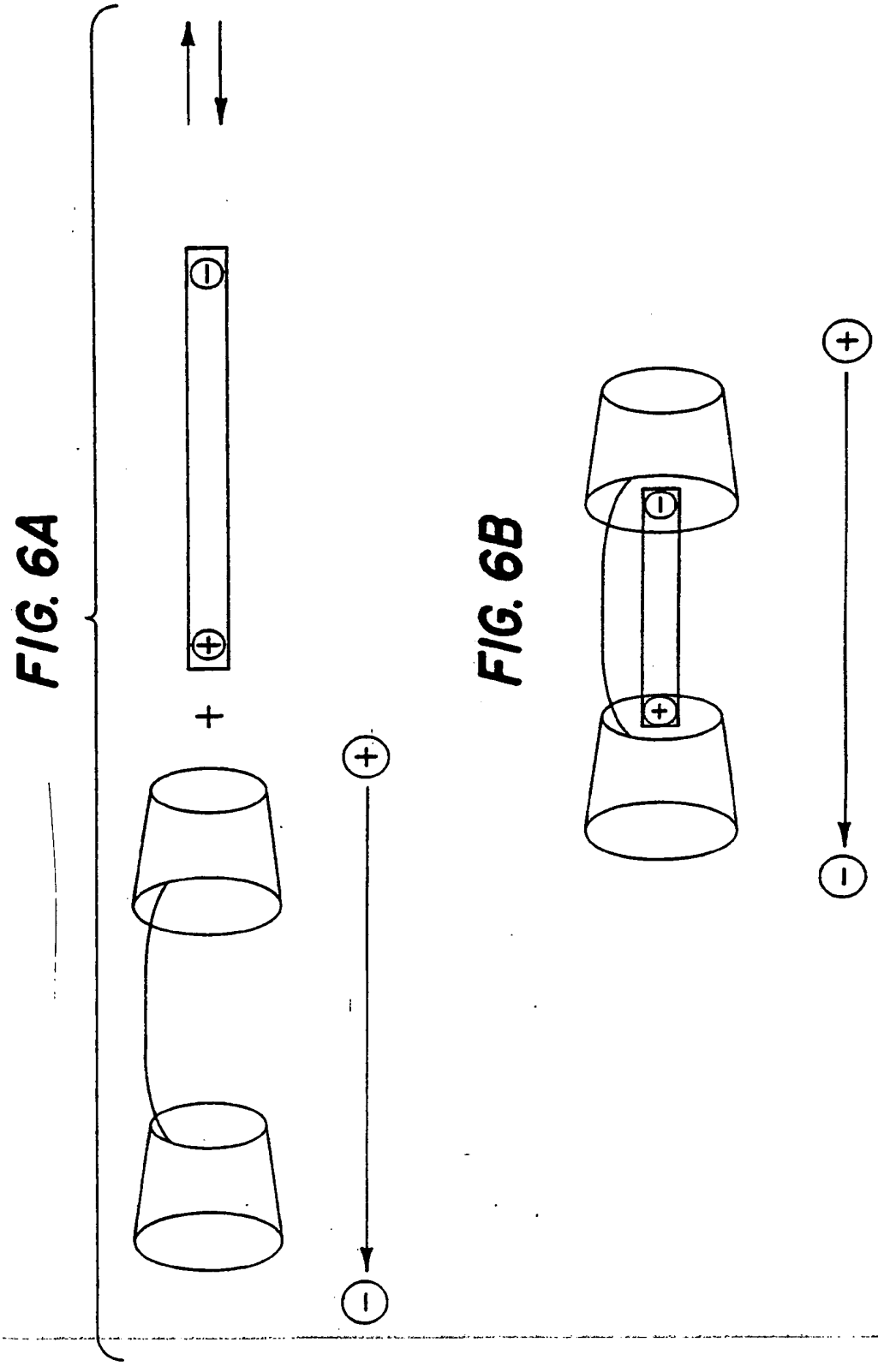


FIG. 6A
FIG. 6B

FIG. 7A

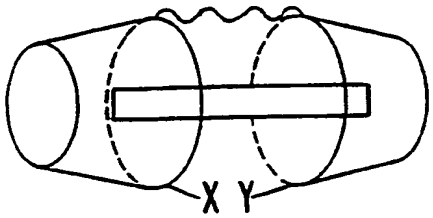


FIG. 7B

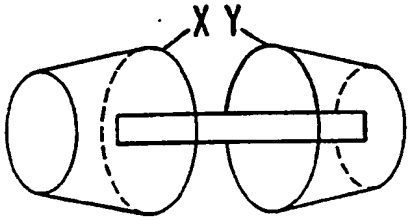


FIG. 7C

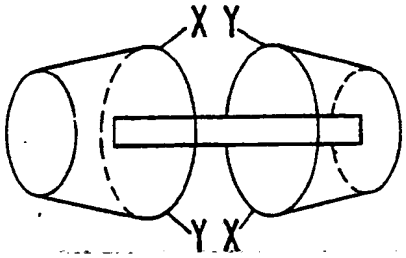


FIG. 8

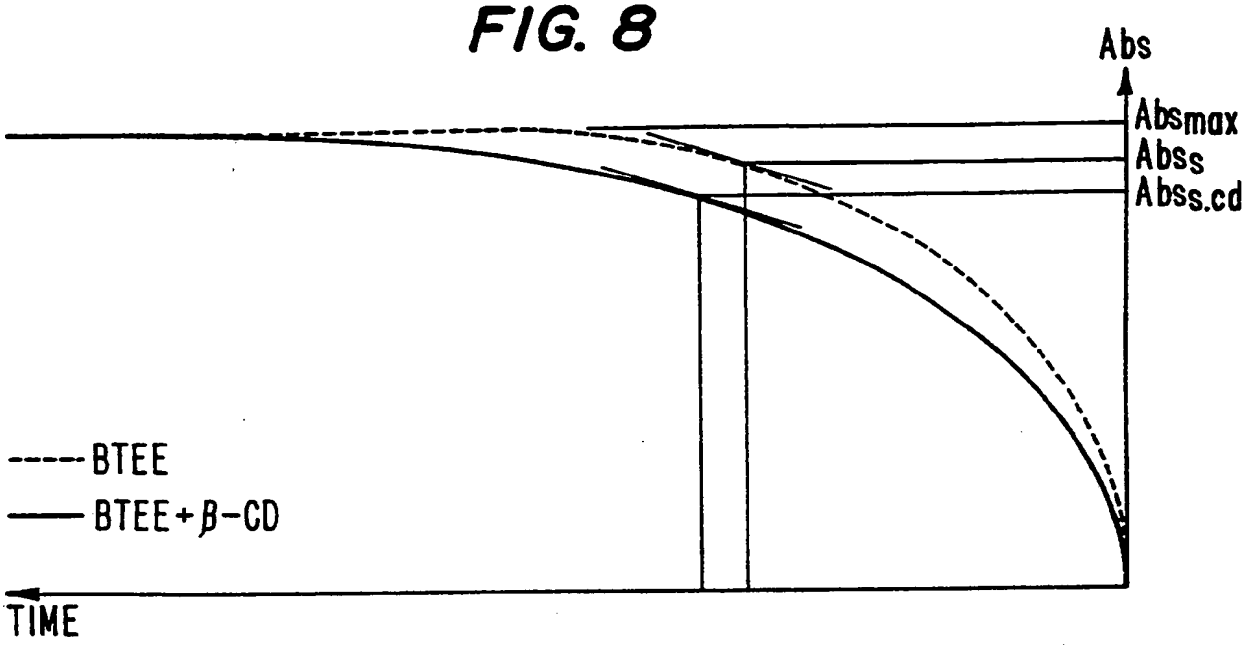

Abs
Absmax
Abss
Abss.cd
----- BTEE
—— BTEE+β-CD
TIME

FIG. 9

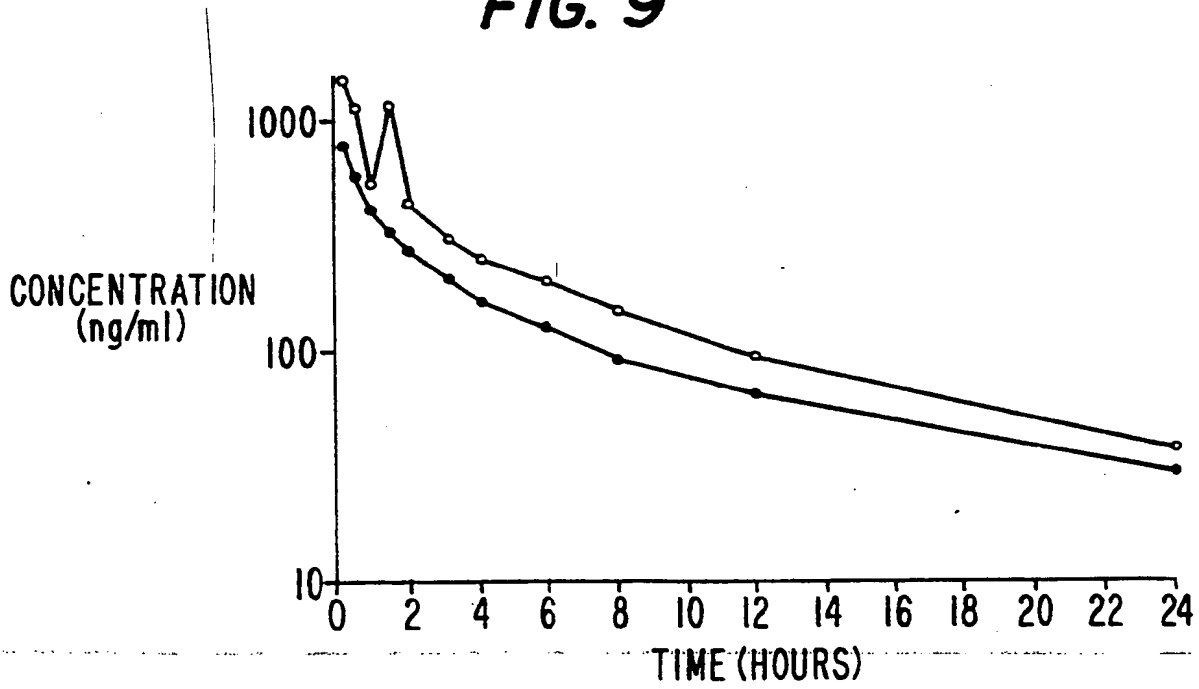

CONCENTRATION (ng/ml)
1000
100
10
0 2 4 6 8 10 12 14 16 18 20 22 24
TIME (HOURS)

FIG. 10

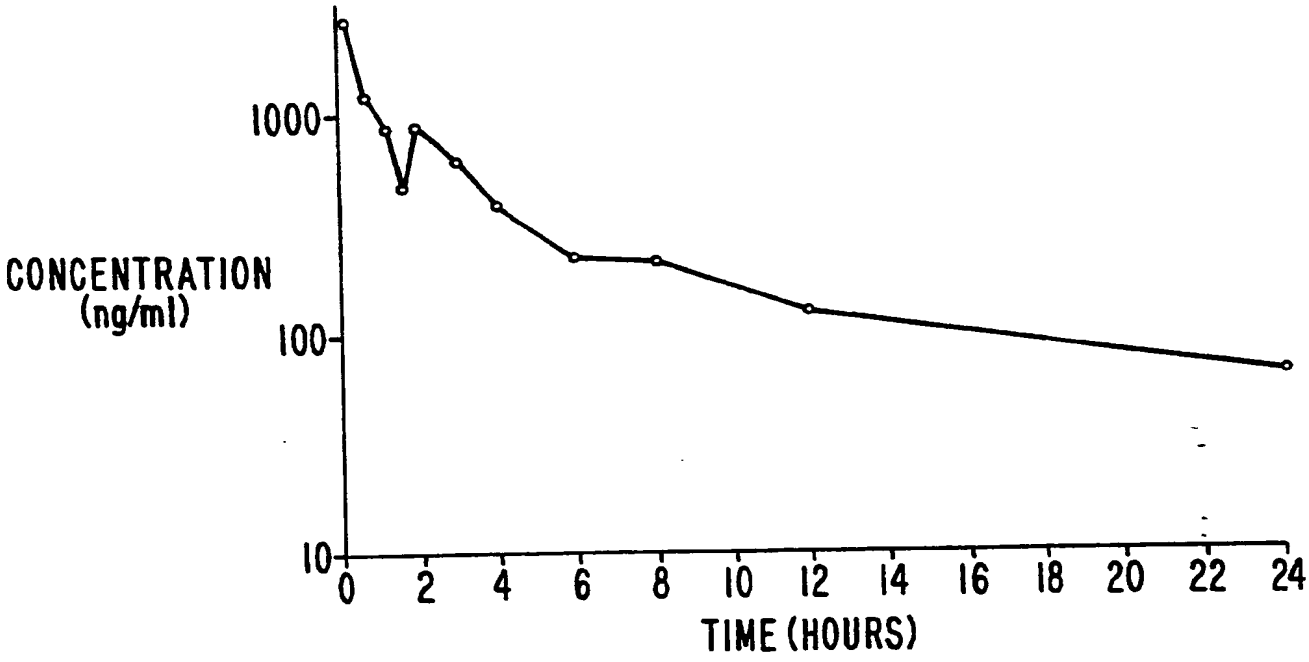
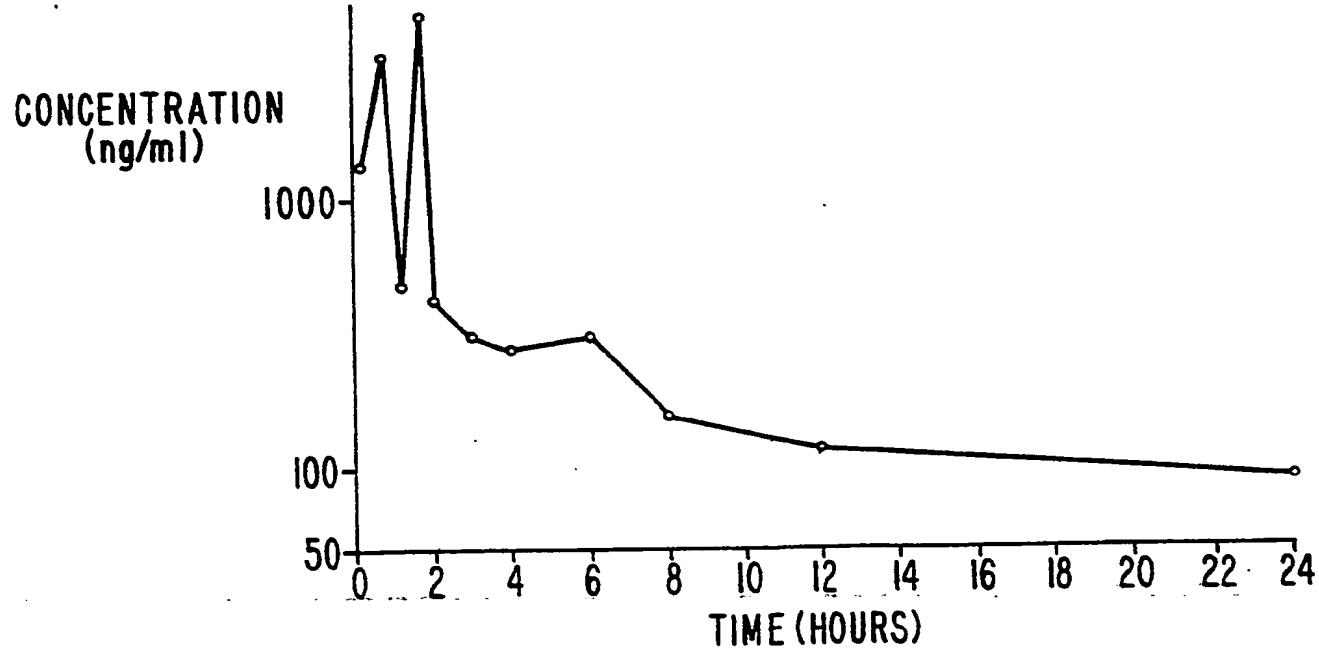

CONCENTRATION
(ng/ml)
1000
100
10
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (HOURS)

FIG. 11


CONCENTRATION
(ng/ml)
1000
100
50
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (HOURS)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 91/00071

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C08B 37/16, C08L 5/16, A61K 47/40 // A01N 25/00

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC	C08B 37/16, C08L 5/16

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

AU : IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X,Y	J. Am. Chem. Soc., Vol 108, No 15 1986; Iwao Tabushi et al; "Artificial Receptors for Amino Acids in Water. Local Environmental Effect on Polar Recognition by 6A-amino-6β-carboxy - and 6β-amino-6A-carboxy- -cyclodextrins; see pages 4514-4518.	(1,29,64)
X,Y	Chem. Pharm. Bull; Vol 35, No 5 , 1987 Akihiko Ueno et al; "Greater Guest Binding Ability of β-cyclodextrin having both a Cap and a Floor". See pages 2151-2154.	(1,29,64)
X,Y	J. Org. Chem, Vol 53, No 9, 1988 Kahee Fujita et al; "Synthesis of $6^A,6^X$-Di-O-(p-tosyl)-γ-cyclodextrins and their Structural Determination through Enzymatic Hydrolysis of $3^A,6^A$; $3^X,6^X$-dianhydro-γ-cyclodextrins". See pages 1943-1947. (continued)	(1)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21 May 1991 (21.05.91)	29 May 1991
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	T. SUMMERS

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X,Y	Tetrahedron Letters, Vol 29, No 12, 1988 (Pergamon Press plc); Iwao Tabushi et al; "Dynamic Molecular Motions of Guest Molecule Included in Modified β-cyclodextrins". See pages 1413-1416.	(1,29,64)
X,Y	EP,A2, 274444 (BRISTOL-MYERS COMPANY) 13 July 1988 (13.07.88)	(1,29,64)
X,Y	AU,B, 38352/85 (565966) (JANSSEN PHARMACEUTICA N.V.) 12 July 1985 (12.07.85)	(1,29,64)
Y	EP,A2, 295476 (EDMOND PHARMA S.R.L.) 21 December 1988 (21.12.88)	(1,29,64)
P,X	WO,A, 90/02141 (AUSTRALIAN COMMERCIAL RESEARCH & DEVELOPMENT LIMITED) 8 March 1991 (08.03.90)	(1,29,64)
X,Y	US,A, 4535152 (SZEJTLI et al) 13 August 1985 (13.08.85)	(1)
X,Y	US,A, 3565887 (PARMERTER et al) 23 February 1971 (23.02.71)	(1)
X,Y	US,A, 4169079 (TABUSHI et al) 25 September 1979 (21.09.79)	(1,29)
X,Y	US,A, 4638058 (BRANDT et al) 20 January 1987 (20.01.87)	(1)
Y	US,A, 4826963 (STADLER et al) 2 May 1989 (02.05.89)	(1,29,64)
X,Y	US,A, 3553191 (PARMERTER et al) 5 January 1971 (05.01.71)	(1)
X,Y	WO,A, 89/06536 (FOLKMAN) 27 July 1989 (27.07.89)	(1,29,64)
Y	WO,A, 90/01320 (AMERICAN MAIZE PRODUCTS COMPANY) 22 February 1990 (22.02.90)	(1,29,64)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [ ] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.[ ] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2.[ ] Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.[ ] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1.[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00071

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
EP 274444	AU 10045/88	JP 63190824	ZA 8800088
AU 38352/85	CA 1222697 EP 149197 NO 853070	DE 3346123 FI 853198 WO 8502767	DK 3595/85 HU 40561 ZA 8410042
EP 295476	IT 8720925 US 5010064	IT 1204725	JP 1093587
WO 9002141	AU 44290/89 PT 91563	CN 1040987	IL 91360
US 4535152	EP 119453	HU 34227	
US 4169079	JP 54061291	JP 54061290	JP 54060761
US 4638058	DE 3345779	EP 147685	JP 60152502
US 4826963	CN 86106889 FI 863652 PL 261349	DD 254939 HU 41759 JP 61239841	EP 214647 JP 62103077

CONTINUED

INTERNATIONAL APPLICATION NO. PCT/AU 91/00071 (CONTINUED)

Patent Document Cited in Search Report		Patent Family Members					
WO	8906536	AU	30327/89	CN	1036135	DK	1713/90
		EP	325199	EP	398925	ES	2017808
		IL	88970	JP	1279828	ZA	8900383
WO	9001320	EP	381747	HU	51287	JP	2167228

END OF ANNEX